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CHEMICAL CONTROL OF BRUSH AND ENVIRONMENTAL SAFETY OF ROADSIDE VEGETATION MANAGEMENT CHEMICALS

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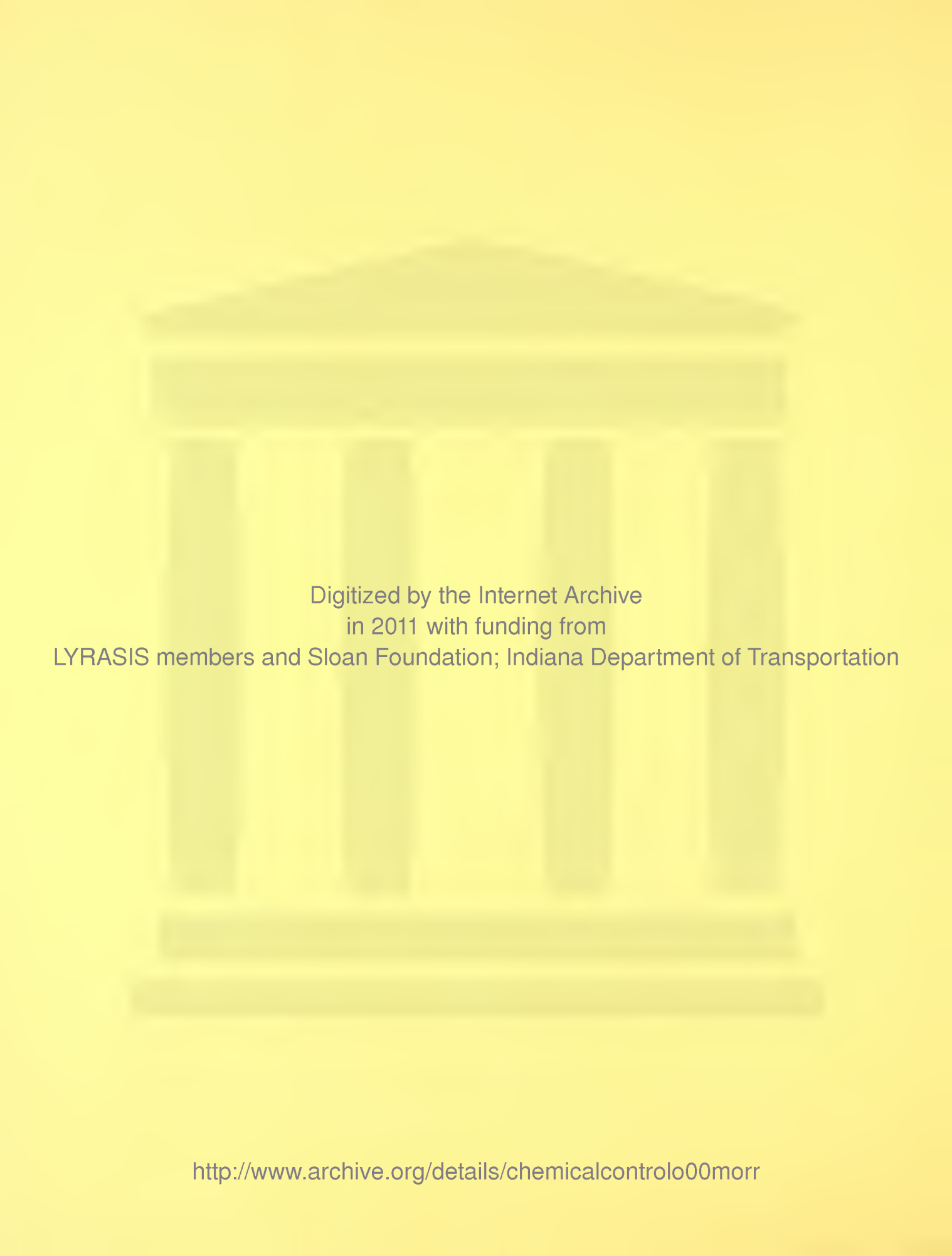
PURDUE UNIVERSITY
INDIANA STATE HIGHWAY COMMISSION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Interim Report

CHEMICAL CONTROL OF BRUSH AND ENVIRONMENTAL SAFETY OF ROADSIDE VEGETATION MANAGEMENT CHEMICALS

TO: J. F. McLaughlin, Director
Joint Highway Research Project

December 1, 1976

Project: C-36-48F

FROM: H. L. Michael, Associate Director
Joint Highway Research Project

File: 9-5-6

The attached Interim Report titled "Chemical Control of Brush and Environmental Safety of Roadside Vegetation Management Chemicals" is submitted on the HPR Part II Research Study titled "Low Cost Maintenance Program for Indiana Roadsides". The Report has been authored by the principal investigator Professor D. James Morre, Department of Biological Sciences.

The roadside chemical weed control program and mowing practice in the state have resulted in substantial roadside area where mowing is not desirable. Brush control is necessary in such areas and with the prohibition of the use of 2,4,5-T, an effective brush control chemical, study was necessary to develop effective means of brush control. The study reported here investigated various chemical combinations and their brush control effectiveness as well as their environmental safety. Recommendations are made as to the brush control agent which should be used in Indiana and as to its application.

The Report upon acceptance by the JHRP Board will be submitted to ISHC and FHWA for review and acceptance as partial fulfillment of the objectives of the research.

Respectfully submitted,

Harold L. Michael

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Interim Report

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by

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and the
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The contents of this report reflect the views of the author who is responsible for the facts and the accuracy of the data presented herein. The contents do not necessarily reflect the official views or policies of the Federal Highway Administration. This report does not constitute a standard, specification, or regulation.

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16. Abstract

The herbicide 2,4,5-T, alone or in combination with 2,4-D (brush killer) either as an ester formulation or as an amine salt, applied either as a dormant treatment in winter or to foliage in summer or early fall, remains as the most effective chemical treatment for control of roadside brush. However, regulatory rulings prohibit the use of 2,4,5-T on home grounds, recreational areas, and in or near water areas. Discontinuance of the use of 2,4,5-T in roadside applications on the basis of environmental safety should be seriously questioned. In the meantime, it became necessary to identify and test other herbicides and herbicide mixtures as alternative brush control agents for inclusion in roadside maintenance programs.

A mixture of equal parts of an amine salt formulation of 2,4-D + 2,4,5-TP(Silvex) + dicamba (Banvel) is recommended for inclusion in the Fall-Spring spraying rotation to be applied by off-road equipment to unmowed portions of the Interstate System. This same mixture or "Krenite" brush control agent are recommended as a foliar application for control of brush along county roads and other situations where hydraulic spray applications directly to stems and foliage are indicated.

Environmental safety of these herbicides was also investigated and is reported. Potential hazards were found to be minimal if the sprays were used as recommended. Control of herbicide drift is an important aspect of any chemical control of brush.

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HIGHLIGHT SUMMARY

The herbicide 2,4,5-T, alone or in combination with 2,4-D (brush killer) either as an ester formulation or as an amine salt, applied either as a dormant treatment in winter or to foliage in summer or early fall, remains as the most effective chemical treatment for control of roadside brush. However, regulatory rulings prohibit the use of 2,4,5-T on home grounds, recreational areas, and in or near water areas. Discontinuance of the use of 2,4,5-T in roadside applications on the basis of environmental safety should be seriously questioned. In the meantime, it became necessary to identify and test other herbicides and herbicide mixtures as alternative brush control agents for inclusion in roadside maintenance programs.

A mixture of equal parts of an amine salt formulation of 2,4-D + 2,4,5-TP (Silvex) + dicamba (Banvel) is recommended for inclusion in the Fall-Spring spraying rotation to be applied by off-road equipment to unmowed portions of the Interstate System. This same mixture or "Krenite" brush control agent are recommended as a foliar application for control of brush along county roads and other situations where hydraulic spray applications directly to stems and foliage are indicated. A 3-way mixture of 2,4-D + 2,4-DP + 2,4,5-TP and a 2-way mixture of 2,4-DP and 2,4,5-TP are still under investigation as possible alternatives to the dicamba (Banvel)-containing 3-way herbicide mixture for use in midsummer applications as amine salts on county roads where injury to soybeans becomes a problem.

Environmental safety of these herbicides to fish, fresh water organisms, and non-target vegetation was also evaluated. Similar evaluations were made with picloram (Tordon) and one agent to control herbicidal drift (Nalco-trol). The three herbicides, 2,4-D, dicamba (Banvel) and picloram (Tordon) as acid or

amine salt formulations exhibited low toxicity to approximately 20 species of fresh water and marine algae even at rates approaching the maximum solubility in water. Ester formulations, including 2,4,5-T or Silvex which is formulated and sold only as an ester, were more toxic. Equivalent results were obtained with fish. For these reasons, only amine salt formulations of 2,4-D or dicamba are recommended and the Silvex content should not exceed more than 1/3 of the total herbicide mixture in the 3-way combination herbicide.

Of the three herbicides tested, only picloram (Tordon) was persistent either in the soil or water. The hazards of this material to non-target vegetation were evaluated using several biological assay procedures developed. Potential hazards were shown to be minimal if the material was applied in the fall but more serious following a spring application. A combination herbicide of 3 parts picloram and 1 part 2,4-D had characteristics similar to picloram alone. Good control of a wide range of brush species was achieved as well as excellent control of MILKWEED and all other broad leaf species including CANADA THISTLE. Attempts to use this material in the Indiana State Highway Program under an experimental label were unsuccessful.

2,4-D alone is of limited effectiveness as a brush control agent. Fall applications of dicamba, either alone or with 2,4-D, were also ineffective in the control of brush.

Control of herbicide drift is an important aspect of any chemical program for control of brush. This is especially important for mid-summer applications to county roads where soybean fields are involved. An appropriate drift control agent or system should be used and wind conditions should be still. Dicamba (Banvel), because of possible injury to soybeans and other crops and non-target vegetation, must be applied either alone or in combination in late fall or early spring after crops have matured or before emergence.

INTRODUCTION

According to the New York Department of Transportation Guide for the Determination of Mowing Limits, safety overrides all other features affecting roadside maintenance. Sight distances must be maintained at intersections and on the insides of curves. Safety setbacks for major trees must be observed. Guard rails, bridge approaches, signs, and other traffic control devices must be kept open to view.

Brush is one of the major offenders in obstructing vision. Reduced mowing practices favor the growth of brush (Fig. 1A). Within two years, black locust, willow or elm will become established in unmowed portions of roadsides. In non-prairie areas, where woody vegetation is natural to the enviornment and a continual invader, one must be prepared for a long fight. Brush grows up into trees which represent solid objects and present even more serious safety hazards (Fig. 1A). Trees too near traffic lanes must be removed usually at considerable expense.

Brush along county roads is a continuing problem. Sight distances on curves are reduced (Fig. 1B), approaches to intersecting roads or lanes are obscured (Fig. 1B), signs and other traffic control devices are concealed (Fig. 1C), and the situation may degenerate to the point that roadside brush becomes a threat to adjacent agricultural land (Fig. 1D).

Brush along roadsides will be controlled one way or another. It can be either as expensive removal of dangerous trees or through low-cost application of chemicals when the brush is small. Newly instituted, cost-saving practices of reduced mowing along Interstates demands that some form of brush control practice be instituted. Use of chemicals is the most convenient and least expensive but which chemicals?

Figure 1. Roadside brush is a serious problem of roadside maintenance. A. Small brush and trees are encouraged by reduced mowing practices and become trees which represent even more serious traffic hazards. B. Brush reduces sight distances on curves (arrow) and may conceal approaches of intersecting roads (double arrows). C. Signs or other traffic control devices may be obscured. D. Brush also poses a threat to adjacent agricultural land.

Figure 2. In non-prairie areas, woody vegetation is natural and is a continual invader in areas which do not receive regular mowing.

In the past, the herbicide 2,4,5-T was used extensively and effectively as a brush control agent along roadsides. It was most generally used in combination with 2,4-D. The herbicide combination decreased the overall cost of the materials with no significant loss or a slight gain in treatment effectiveness. However, regulatory rulings now prohibit the use of 2,4,5-T on home grounds, recreational areas, and in or near water areas. The purpose of this research project was to identify and test a suitable herbicide treatment program for use on the Interstate System in combination with reduced mowing practices that would provide a practical alternative to 2,4,5-T and at the same time control braod-leaf weeds. This report provides one such treatment in the form of a specific recommendation.

Since environmental safety is a major factor in the design of any vegetation management practice, much of the body of this report is devoted to tests designed to determine or verify safety of the recommended materials to man, domestic animals, fish, aquatic food chain organisms, and nontarget vegetation. Additionally, test results are reported for picloram (Tordon), a picloram + 2,4-D mixture, and "krenite" brush control agent. The latter are not yet recommended for use along the Interstate System but may find applications as spot treatments or for brush control along county roads.

SUMMARY OF BRUSH CONTROL TREATMENTS FOR USE OTHER THAN FOR HOME GROUNDS,

RECREATIONAL AREAS, OR IN OR NEAR WATER AREAS

FOLIAGE-STEM SPRAY: 2,4,5-T or 2,4-D + 2,4,5-T amines or low volatile esters at a rate of ½ - 1 gal (2-4 lbs) herbicide in 100 gallons water. Note: If sprayed directly over water, ester formulations should not be used to avoid fish kills.	Wet all leaves, stems and suckers thoroughly to the groundline. Apply any time after leaves reach full size until about 3 weeks before frost. Avoid drift.
CUT STUMP: 2,4,5-T or 2,4-D + 2,4,5-T low volatile esters or oil soluble animes at a rate of 12 - 16 pounds herbicide in 100 gallons diesl or fuel oil.	To prevent stumps from sprouting, thoroughly drench each stump or cut surface with the herbicide mixture in oil. Apply any time of year as soon as possible after trees are cut.
BASAL BARK: 2,4,5-T or 2,4-D + 2,4,5-T low volatile esters or oil-soluble amines at a rate of 8-32 pounds of herbicide in 100 gallons diesel or fuel oil.	Most useful for small trees, less than 4 inches in diameter. Apply as a basal spray directed to trunk or stem from ground line up 12-20 inches. Wet root collar and stem portion thoroughly. May be used year around but is especially effective as a dormant treatment.
FRILL OR GIRDLE: 2,4,5-T or 2,4-D + 2,4,5-T low volatile esters or oil soluble amines at a rate of 12 - 16 pounds of herbicide in 100 gallons diesel or fuel oil.	Cut overlapping notches with an ax or continuous ring with chain saw. Fill the notches with as much herbicide mixture as possible or drench the cut surfaces of the saw girdle. Apply any time of year as soon as possible after cuts are made. Recommended for trees larger than 5-6 inches diameter.
INJECTION: 2,4,5-T or 2,4-D + 2,4,5-T low volatile esters at a rate of 4 lbs herbicide in 20 gallons oil.	Spaced cuts at 1-3 inch intervals are made around tree. The herbicide oil mixtures are placed in the cuts. Various mechanical devices and herbicide formulations are available for this method of treatment. Check manufacturers instructions for any particular method or product.

RESTRICTION: Do not apply 2,4,5-T on home grounds, recreational areas or in or near water areas.

THE TOXICITY OF 2,4-D AND PICLORAM HERBICIDES TO FISH

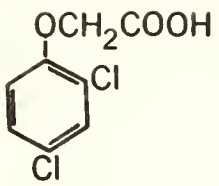
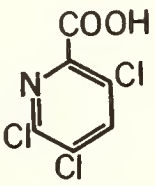
Introduction

Although considerable information is available from chemical manufacturers, the environmental protection agencies, and toxicity testing laboratories attesting to the non-toxicity of 2,4-D to fish, I believed it advisable to supplant this information with some first-hand observations. Additionally, a newer material called picloram or Tordon herbicide (Table 1) was also included in these tests as a possible replacement for 2,4,5-T in herbicide mixtures. The mode of action of picloram is similar to that of 2,4-D (4, 5, 6, 8, 16, 19, 21) but has a greater herbicidal effectiveness due to increased mobility and resistance to breakdown within the plant (6). As a consequence of its resistance to breakdown, picloram introduced into the biosphere may persist for a year or more (7, 9. 12, 18) in contrast to 2,4-D and other herbicides which are broken down more rapidly (11). This study deals with the toxicity of 2,4-D and picloram-containing herbicides to green sunfish and other fish species.

Materials and Methods

Green sunfish (Lepomis cyanellus), 50-150 g, were maintained in pond water at 24° C with continuous aeration and on artificial diets. Rainbow trout (Salmo gairdnerii) were obtained from the Silver Creek Trout Farm, Route 5, Topash Road, Dowagiac, Michigan.

Table 1. Characteristics of 2,4-D and picloram derivatives.

Compound	Abbreviation	Chemical Structure[1]
2,4-Dichloro-phenoxyacetic acid	2,4-D	
4-Amino-3,5,6-trichloropicolinic acid	Picloram (Tordon[2])	

[1] Acid (R=H); salt (R=metal ion or organic amine); ester (R=long chain alcohol).

[2] Registered trademark of the Dow Chemical Company.

Table 2. Effect of herbicide formulations on the swimming response of green sunfish.

Herbicide	Concentration	Formulation	Acid Equivalent	Average Response Time
2,4-D	$5X10^{-4}$ M	Acid	99%	No effect in 41 hr
		Li+Salt	95%	No effect in 41 hr
		Isopropyl-Diethylanolamine Salt	28%	No effect in 41 hr
		Butoxyethanol Ester	43%	60 min
Picloram	$5X10^{-4}$ M	Acid	99%	No effect in 41 hr
		Technical	91%	5 min
		K+Salt	22%	5 min

Trout were maintained in 84" X 24" X 22" fiberglass tanks equipped with automatic cooling and filtration systems ("Living Stream" units; Frigid Units, Inc., 3214 Sylvania Avenue, Toledo, Ohio). The fish were fed twice daily using Master Mix Trout Food and the water temperature was maintained between 13 and 15° C.

In each experiment, fish mortality was recorded and at the end of the study, fish were examined for tissue abnormalities. The livers were excised, weighed and sampled for histological examination.

For electron microscopy, portions of the livers were fixed at 0-4° in 2.5% glutaraldehyde (Fisher, Biological Grade) in 0.1 M sodium phosphate at pH 7.2 followed by a buffer rinse and post fixation in 1% osmium tetroxide in the same buffer. Specimens were dehydrated in a graded acetone series and embedded in Epon (24). Thin sections were post-stained with lead citrate (22) after being mounted on carbon-coated parlodion-covered grids and were viewed with a Philips EM-300.

Results

With 2,4-D, neither the acid nor commercial salt formulations were toxic to green sunfish at a concentration of 5×10^{-4} M (110 ppm acid equivalent) (Table 2). However, at this same concentration, the butoxyethanol ester of 2,4-D proved toxic after 60 minutes of exposure. Similar results were reported previously by Butler (1) (Table 3) in tests using three salt water species of fish and with rainbow trout (Table 4). With picloram, the 99%

Table 3. Comparative toxicity of 2,4-D formulations to fish (*Leiostomus xanthurus*, *Fundulus similus* and *Mugil cephalus*)[1]

Formulation	LC_{50}—ppm ae 48 hr Exposure
Acid	no effect at 50 ppm
Dimethylamine salt	no effect at 15 ppm
2-Ethylhexyl ester	no effect at 10 ppm
Propyleneglycolbutylether ester	4.5
Butoxyethanol ester	5.0

[1] From Butler (1).

Table 4. Comparative toxicity of picloram formulations to rainbow trout (*Salmo gairdnerii*)[1]

Formulation	Equivalent	(LC_{50}—ppm ae) Exposure Time	
		24 hr	96 hr
Triisopropanolamine salt	55.8%	279	209
Triethylamine salt	70.5%	43	29
Isooctyl ester	68.3%	10	3

[1] From E. E. Kenaga (17).

analytical grade material was also nontoxic at 5×10^{-4} M (120 ppm acid equivalent). However, both the 91% technical picloram and the 22% commercial formulation were toxic (Table 2) suggesting the presence of an impurity in these preparations. Similar toxicities of picloram to fish were reported by Kenaga for rainbow trout and other species (17) with the isooctyl ester being at least 10 times more toxic than the corresponding amine salts (Table 4).

In our studies, fish were quickly immobilized by 5×10^{-4} M technical picloram but did not die. Fish treated for up to one hour with this concentration of technical picloram recovered motility and swam normally upon return to pond water without herbicide. The recovery time varied as an approximately linear function of the treatment time (Fig. 3). After recovery, the fish were then given a second exposure to technical picloram. After the second exposure and return to pond water without herbicide, the fish again began to swim normally but the recovery times were generally shorter (Fig. 3) than after the first exposure. This trend continued through a third exposure to technical picloram and after a fourth exposure many of the fish failed to even respond, i.e. 0 recovery time.

Liver changes were observed even in those fish exposed to 10^{-4} M technical picloram (Table 5), a concentration which did not affect the swimming response. Ultrastructural changes associated with exposure to technical picloram included the disappearance of rough-surfaced endoplasmic reticulum sheets which were observed in all hepatocytes (liver cells) from untreated fish (Fig. 4) and a conspicuous increase in a tubular or vesicular smooth (lacking ribosomes) form of the endoplasmic reticulum (Fig. 5). Treatment of fish for periods of

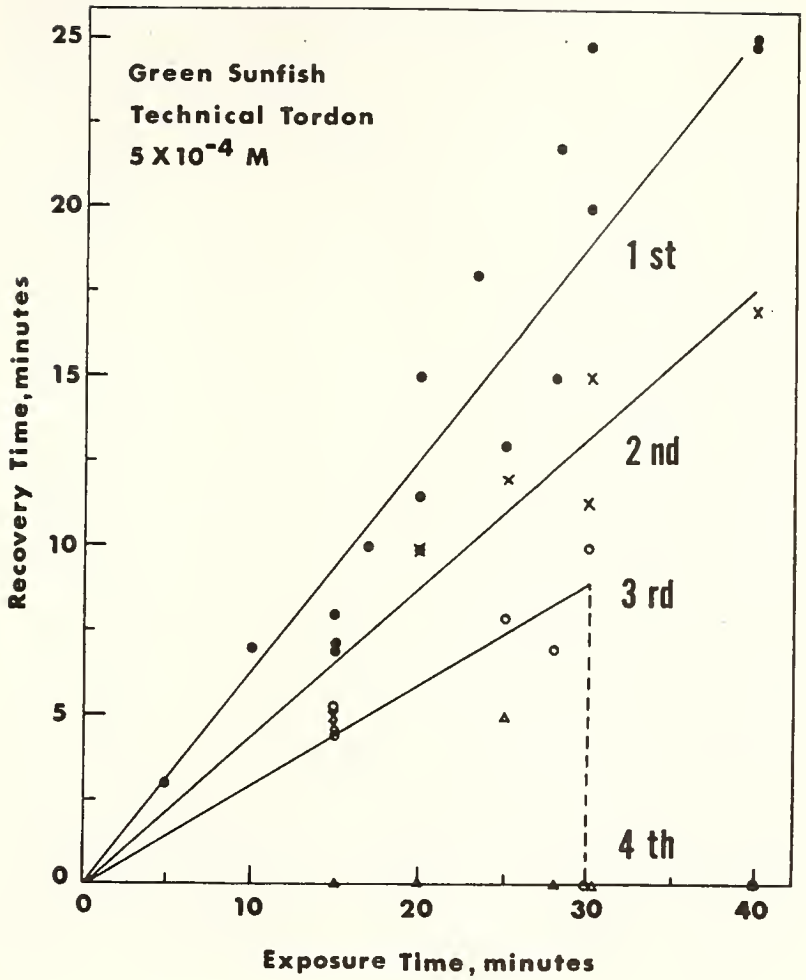


Figure 3. The relationship between exposure time to 5 X 10^{-4} M technical picloram (91% acid) and subsequent recovery of normal swimming response of green sunfish (Lepomis cyanellus).

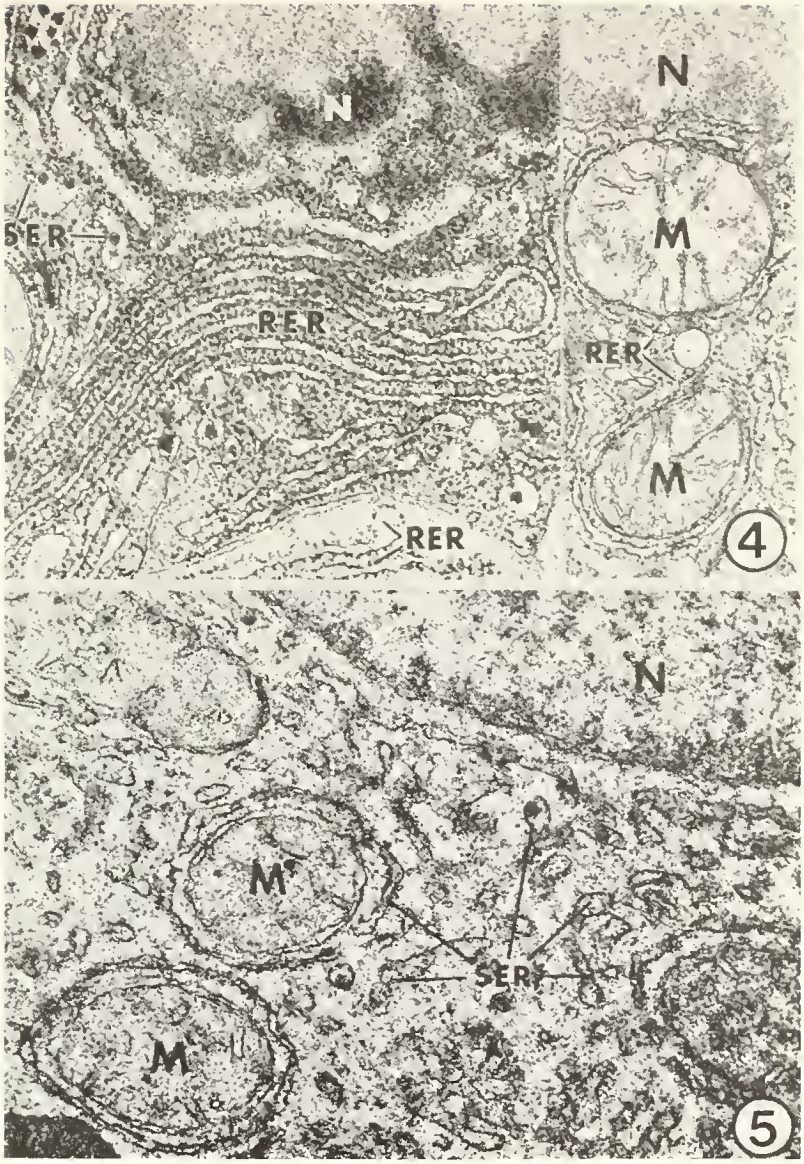


Figure 4. Electron micrographs of fish (Lepomis cyanellus) liver cells. Of interest are endoplasmic reticulum profiles, most of them rough-surfaced (covered with ribosomes) in stacked or whorled arrays (RER) interspersed with short tubular or vesicular profiles of smooth endoplasmic reticulum (SER) (Left X 36,000). Most, if not all, mitochondria (M) of fish hepatocytes are surrounded by lamellae of rough-surfaced endoplasmic reticulum (Right X 32,000). N=nucleus.

Figure 5. Electron micrograph of a fish liver cell (cont. next page)

after 24 hours exposure to 10^{-4} M (91% acid) technical picloram. The large sheets of rough endoplasmic reticulum are absent and in their place are expanses of a tubular or vesicular network of smooth endoplasmic reticulum (SER). Ribosomes are lost from most of the endoplasmic reticulum around the mitochondria (M) as well, especially from the cytoplasmic surface. N=nucleus. X 35,000.

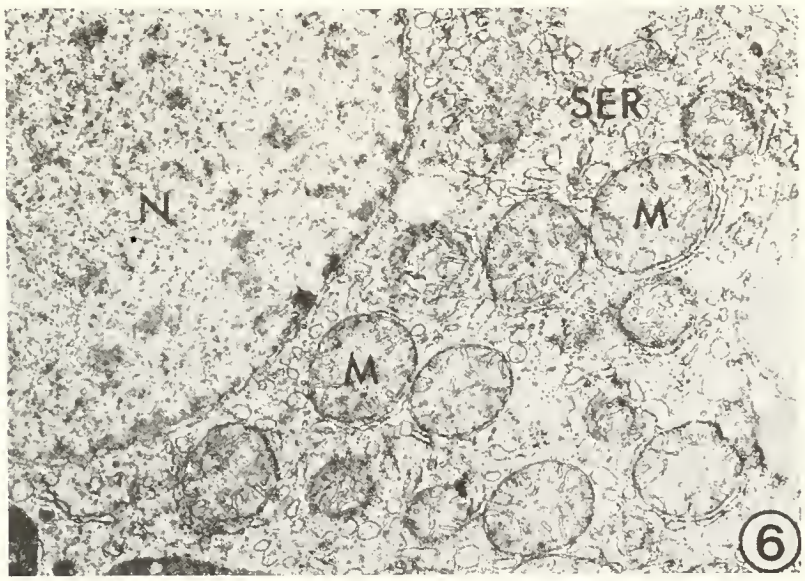


Figure 6. Electron micrograph of a fish liver cell after 5 days of exposure to 10^{-4} M technical (91%) picloram. Most of the endoplasmic reticulum (SER) is smooth-surfaced and vesiculated. Mitochondria (M) and nuclei (N) appear unchanged from 1 day of exposure to technical picloram but considerable variation was encountered from cell to cell. The appearance of hepatocytes examined after 2, 3, 4 and 5 days of exposure to this concentration of technical picloram were similar. X 13,700.

Table 5. Effect of technical picloram (91%) on liver weight of green sunfish (Lepomis cyanellus)

Treatment	Weight of Liver, % of Body Weight[1]	
	5 Days	9 Days
Control	0.65 ± 0.28	0.56 ± 0.16
Technical picloram (91% acid) 10^{-4}M	1.36 ± 0.61	1.08 ± 0.36

[1] Averages from 10 fish ± standard deviation.

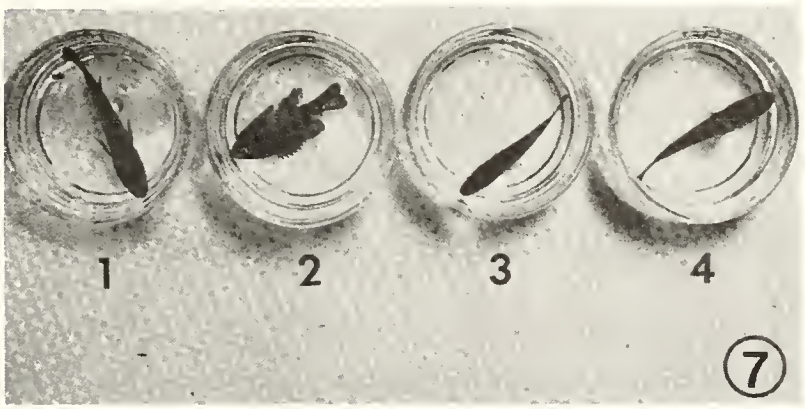


Figure 7. Green sunfish (Lepomis cyanellus) representative of the fish used in this study following treatment with various impurities from technical picloram (see text). Impurity 2 was toxic. In solutions containing the other three compounds (all at 10^{-4} M concentrations), the fish maintained a normal swimming response. 1/5 actual size.

up to 5 days at this same concentration of technical picloram did not result in significant changes in the ultrastructural pattern (Fig. 6) from that observed after 1 day (Fig. 5).

Other significant changes were observed in the Golgi apparatus of liver cells of fish treated with 10^{-4} M technical picloram (91%) acid. In normal fish liver cells, Golgi apparatus of considerable complexity were observed (Fig. 8). The numerous, closely spaced dictyosomes were compactly arranged into a distinct Golgi apparatus zone with secretory vesicles at one or both faces. The Golgi apparatus sometimes appeared staight, curved or cylindrical as shown in Figure 9. In Figure 8, the Golgi apparatus is in the form of a hollow cylinder with the system of interassociated dictyosomes forming the wall of the cylinder (see Fig. 9C).

Livers of fish treated for 24 hours with 5×10^{-4} M picloram were characterized by Golgi apparatus consisting of discrete dictyosomes (Fig. 9A), frequently widely separated, within the Golgi apparatus zone (Fig. 10). Frequently the dictyosome cisternae were curled or in the form of rings.

As a first step in determining the nature of the toxic impurity present in technical picloram, the following compounds were obtained through the courtesy of the Dow Chemical Company, Midland, Michigan:

1) 4-amino-3,5,6-trichloropicolinonitrile
2) 2-(3,4,5,6-tetrachloro-2-pyridyl) guanidine
3) 4-amino-2,3,5,6-tetrachloropyridine
4) 6-amino-3,4,5-trichloropicolinic acid

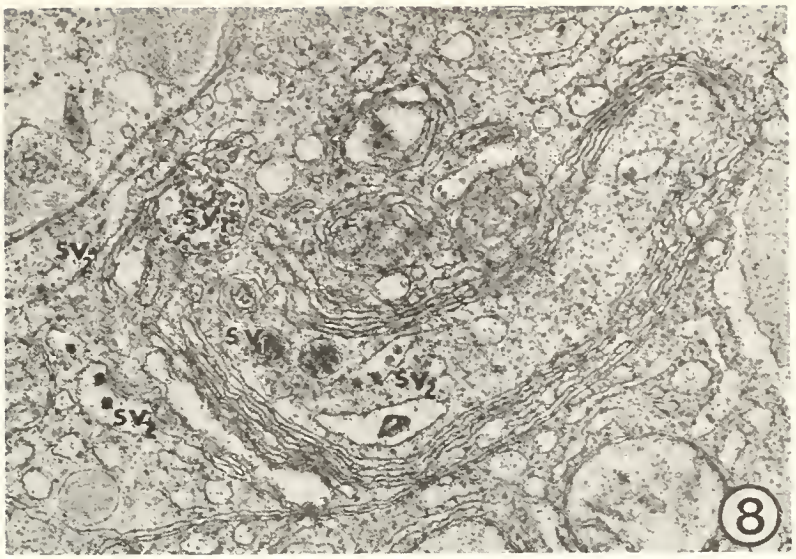


Figure 8. Electron micrograph of a portion of the Golgi apparatus from the liver of a control fish showing the compact arrangement of dictyosomes in cylindrical array (Compare with Fig. 9C). Two types of secretory vesicles are illustrated. One has a compact appearance with many small, dark-staining particles and often a dark-staining matrix (sv_1). The other has a swollen appearance with scattered dark-staining particles of larger diameter and a non-staining matrix (sv_2). X 32,300.

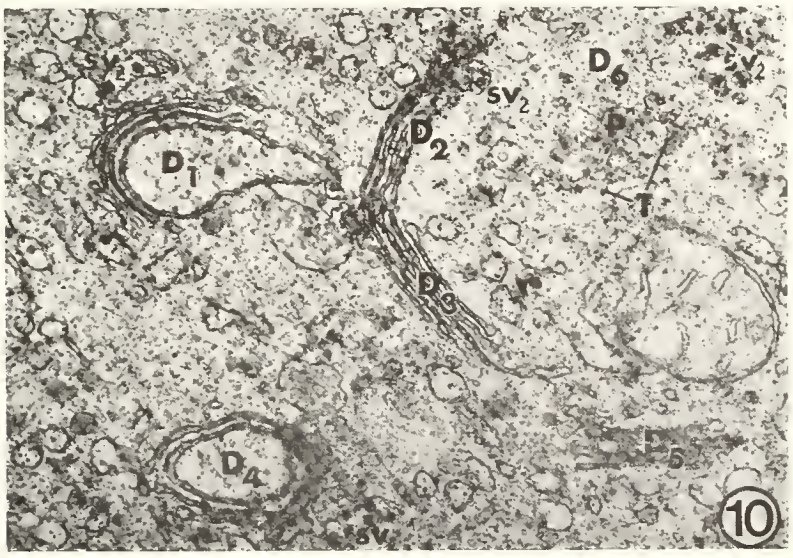


Figure 10. Electron micrograph of a portion of the Golgi apparatus from the liver of a fish exposed for 24 hours to 10^{-4} M technical picloram (91% acid) showing the dispersed arrangement of dictyosomes (D_1-D_6). D_6 is sectioned tangentially and reveals a portion of a cisterna in face view with a central plate-like region (P) and tubules (T) at the periphery of the plate or "saccule". Lipoprotein particles are restricted to elements of the smooth reticulum and the swollen vesicles (sv_2) which contain the large lipoprotein particles. X 33,500.

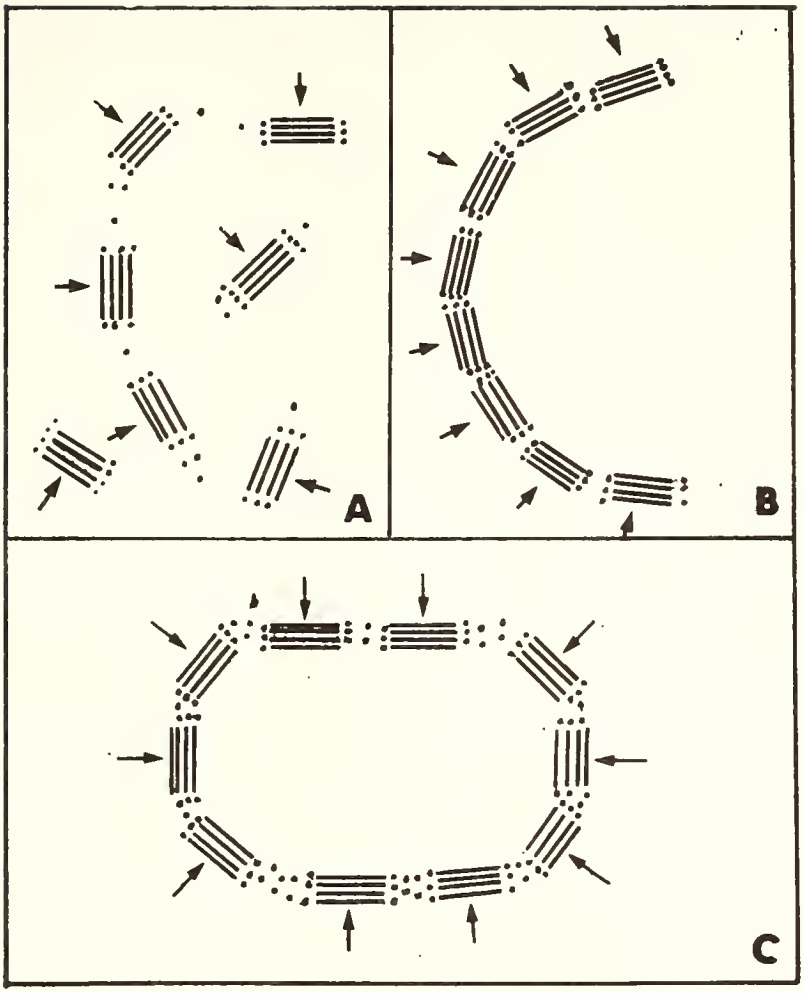
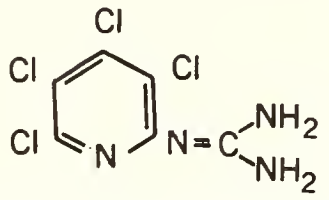


Figure 9. Diagram illustrating principal forms of the Golgi apparatus in normal and picloram-treated fish livers. Arrows indicate individual dictyosomes (stacks of plate-like regions of Golgi apparatus cisternae, the "saccules", interrupted by discontinuous tubular or fenestrated (with holes) regions). A. The dictyosomes are dispersed as is characteristic of plants, invertebrates, and some animal germ cells. B. The dictyosomes are compactly arranged to form a localized curved array as is characteristic of most somatic animal cells. C. A variation of the compact form of the Golgi apparatus in which the dictyosomes are arranged in a cylindrical array.

These compounds were known to be present in technical picloram as impurities and Compound 2, illustrated below, was toxic to fish (Fig. 7).



2-(3,4,5,6-tetrachloro-2-pyridyl) guanidine

The other 3 impurities tested were not toxic to fish at a concentration of 10^{-4} M (Fig. 7) and the swimming response of the fish in the presence of the compounds was normal.

Discussion

With its relatively low toxicity of 8,200 mg/kg and its rapid excretial rate from the animal body, picloram or Tordon Herbicide seems to present no serious hazard to man or terrestrial animals (17). 2,4-D is even less toxic. The toxicities of these herbicides to birds has been reported to be low (17) and no effect on reproduction of either birds or fish have been noted (17). As summarized in this report, the acute toxicity of picloram or 2,4-D to fish is also very low. However, the use of ester formulations tends to increase toxicity emphasizing the need for testing of specific commercial formulations, combinations of herbicides, and combinations of herbicides with other types of water pollutants.

Pure picloram seems to be virtually without effect on fish. The fact that commercial picloram or Tordon Herbicide has an LD_{50} to fish in the range 10-500 ppm (depending on species, size, and conditions of exposure) seems to arise from a toxic impurity present in technical and commercial picloram formulations. At concentrations at or near the LD_{50}, the loss of swimming response is reversible and upon repeated exposure, the fish are able to adapt to the herbicide. The subacute response is accompanied by liver enlargement, loss of large sheets of rough-surfaced endoplasmic reticulum, and the appearance of a vesicular or tubular smooth form of endoplasmic reticulum. The Golgi apparatus changes from a compact to a widely dispersed arrangement of dictyosomes.

Proliferation of smooth endoplasmic reticulum is a phenomenon commonly associated with the response of liver cells to a variety of drugs and pesticides (2, 15, 20) and with the induction of relatively non-specific steroid and drug-hydroxylating enzymes. The action of these enzymes of the smooth endoplasmic reticulum is to make the drugs more soluble in water and more readily excreted from the organism.

The response of the endoplasmic reticulum of fish liver to technical picloram reported here is different. It involves disappearance of the rough-surfaced sheets of endoplasmic reticulum. It appears that the smooth endoplasmic reticulum of the livers from fish exposed to technical picloram is derived from rough-surfaced endoplasmic reticulum through loss of ribosomes and vesiculation. This interpretation is supported

by the observation that the lamellae of rough-surfaced endoplasmic reticulum which envelope most of the mitochondria also lose ribosomes and appear smooth-surfaced in the treated cells.

Again, the liver changes were studied only with the technical picloram and may very well be a response to one or more of the impurities present rather than to the actual herbicide.

It has been the practice of some investigators and many field personell to regard all formulations of 2,4-D or picloram as equal in phytotoxicity and toxicity to fish. It is apparent from our data and the data of others (1, 3, 13, 14, 17) that the formulation used is important. In shallow water, the amount of an ester formulation required to secure optimum results for brush control (Fig. 11) may result in dramatic kill of fish, not from the basic herbicide, but the manner in which it is prepared for commercial use. However, as illustrated in Figure 11, the concentrations of salt formulations of even technical picloram which are toxic to fish approach the maximum solubility of picloram acid in water and are at least an order of magnitude greater (10-fold) than those which might be expected from accidental or direct contamination of lakes or streams through normal use practices. On the same basis, the toxic concentrations reported in the study are 100-100 fold higher than what might be expected in terms of water pollution resulting from runoff of treated soil or vegetation during the spraying of agricultural ditch banks. Consequently, these herbicides present a low potential hazard to fish from normal agricultural or industrial use practices. Amine salts of 2,4-D are considered completely safe.

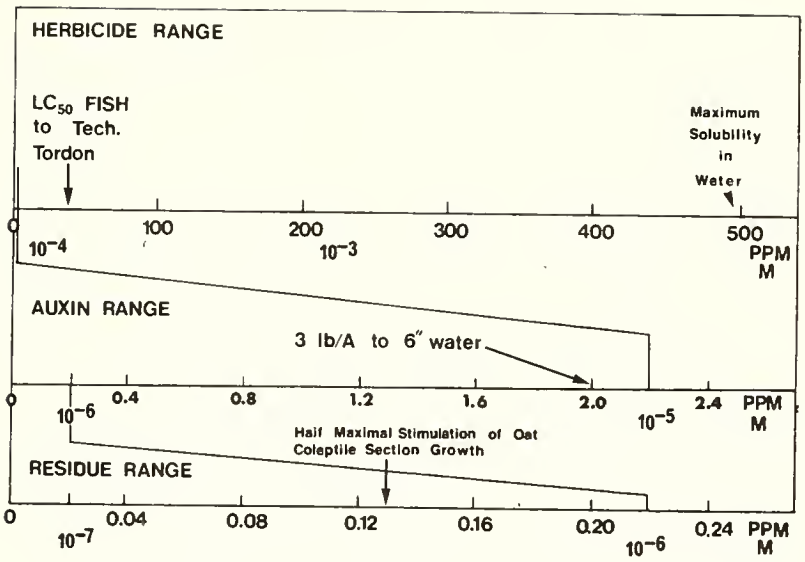


Figure 11. Diagram illustrating some biological responses to 2,4-D and picloram herbicides over the range 0 to 3 X 10^{-3} M. The midportion of the diagram shows an expansion of the range 0 to 10^{-5} M and the bottom scale is an expansion of the range 0 to 10^{-6} M.

Summary

Two common herbicides, picloram or Tordon (4-amino-3,5,6-trichloropicolinic acid) and 2,4-D (2,4-dichlorophenoxyacetic acid) and their salts exhibit low toxicity to fish. Certain formulated derivatives (especially esters) tend to be more toxic than acid salts as is an impurity from technical picloram. Even with picloram containing impurities, adaptive and/or detoxification responses by the fish were demonstrated. These herbicides (picloram and 2,4-D) seem to present a low potential hazard to fish from normal agricultural or industrial practice. 2,4-D appears completely safe.

Acknowledgements

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TOXICITY OF 2,4-D AND PICLORAM TO FRESH AND SALT WATER ALGAE

Introduction

The potential hazard of picloram herbicides (1-6) or of 2,4-D (1) to fresh water algae or phytoplankton from terrestrial runoff water or direct accidental contamination of water has received virtually no attention, except from the popular press. A single report (4) describes experiments in which 1 ppm of picloram did not retard algal growth. Similarly results with other growth regulating agents on growth of algae are limited.

This section of the report summarized information on the effects of 2,4-D and picloram on growth and development of several species of freshwater and marine algae. Such information is required to evaluate the potential threat, if any, of growth regulating agents used for brush control to our environment. Without such information, potentially useful agricultural chemicals may be lost through public or regulatory over-reaction. Alternatively, herbicides may be already entering our lakes, streams and oceans at levels dangerous to their productivity.

Over the past few years, much concern has been generated by "environmentalists" concerning harmful effects of 2,4-D, 2,4,5-T, picloram and related herbicides both from the standpoint of human health and welfare to long-range ecological effects. One newspaper account quoted an unnamed scientist as speculating that if a super-

tanker filled with herbicide concentrate, for example, were separated from its cargo in mid-ocean, all the phytoplankton in a body of water the size of the Indian Ocean would be killed. If this statement were true (such herbicides were apparently and may still be shipped by tanker), the devastating effects of just one accident on the world's oxygen supply would be of considerable consequence.

Materials and Methods

Picloram (4-amino-3,5,6-trichloropicolinic acid, 99% analytical grade), technical picloram (4-amino-3,5,6-trichloropicolinic acid, 91% technical grade) or 2,4-D (2,4-dichlorophenoxyacetic acid, 99% biological grade) were added directly to the growth media at 10^{-3}, 5×10^{-4}, and 10^{-4} M concentrations. The genera examined are summarized in Table 6. All were fresh water organisms except for the marine Chrysophyta Coccolithus and Pleurochrysis. Fresh water algae were grown on at least one optimal medium (7) as well as on at least one minimal medium (soil water, pond water or both) under fluorescent lights (150 ft-c) on a 16 hr photoperiod with gentle shaking and at about 25° C. Marine organisms were cultured in an equal mixture of filtered sea water and soil water.

Growth was determined from changes in optical density at 660 mμ or from cell counts. Cells were examined with the light microscope for morphological changes and, if morphological changes were detected, with the electron microscope.

Table 6. Algal genera tested

Division Chlorophyta

Order Volvocales

Chlamydomonas

Pandorina

Volvox

Order Chlorococcales

Chlorella

Pediastrum

Hydrodictyon

Division Chlorophyta

Order Cladophorales

Pitophora

Order Zygnematales

Spirogyra

Zygnema

Division Chrysophyta

Coccolithus

Navicula

Pleurochrysis

Division Cyanophyta

Chroococcus

Cylindospermum

Merismopedia

Microcoleus

Nostoc

Oscillatoria

Division Euglenophyta

Euglena

Results and Discussion

The solubility of 2,4-D acid in water is approximately 2.5 X 10^{-3} M, that of picloram, 1.8 X 10^{-3} M. Therefore, from a practical standpoint the highest concentration of herbicide tested was 10^{-3} M (220 ppm 2,4-D or 240 ppm picloram). Even at this concentration, we could observe no lasting effect on most of the organisms with either 2,4-D or picloram. In experiments where inhibition of growth was observed at 10^{-3} M, growth was normal at 10^{-4} M. Many of the organisms were cultured for several weeks in the presence of the herbicides and 1 week was the minimum tested.

In the presence of technical picloram, non-motile species were generally less affected than motile species. Motile species were found to lose motility at 10^{-4} M and 5 X 10^{-4} M but not at 10^{-4} M (24 ppm). Data for Euglena gracilis Klebs are given in Table 7. An impurity in the technical picloram, tentatively identified as 2-(3, 4, 5, 6-tetrachloro-2-pyridyl) guanidine (see p. 18), is the major toxic principle.

Even with technical picloram, not all organisms were affected. Pediastrum, for example, grew on technical picloram even at the highest concentrations. In the declining phase of the culture, more colonies were found in the presence of picloram than in its absence or in the presence of 2,4-D (Table 8). Picloram acid (99%) was generally non-toxic.

The results show that the potential hazard of 2,4-D or picloram to both fresh water and marine algae is nil from terrestrial runoff

Table 7. Effect of herbicides on Euglena gracilis Z strain growing in soil water after 3 days.

Herbicide	Conc.	O.D. 660 mμ	% motile cells
None	-	0.26	100
2,4-D acid (99%)	10^{-3} M	0.27	100
Analytical picloram (99% acid)	10^{-3} M	0.26	100
Technical picloram (91% acid)	10^{-3} M	0.23	5
	5×10^{-4} M	0.23	10
	10^{-4} M	0.26	100

Table 8. Effects of herbicides on viability of Pediastrum in pond water.

		Cells/ml ± standard deviation	
Herbicide	Conc.	After 1 doubling	Two weeks later
None	-	114 ± 14	26 ± 8
2,4-D acid (99%)	10^{-4} M	104 ± 10	26 ± 10
Technical picloram (91% acid)	10^{-4} M	112 ± 16	60 ± 10

water (concentrations of 0.1 ppm or less) or direct or accidental contamination (3 lb/A applied directly to 6 inches of water gives a concentration of about 2 ppm). Further studies will be necessary to determine what effects these herbicides might have on subtle ecological shifts as well as to assess the effect of herbicides in combination with other water pollutants. 2,4-D derivatives (particularly esters) may be substantially more toxic than the parent acid (1). Salt formulations are much less toxic than the esters. There is no evidence for biological magnification of either 2,4-D or picloram in algae (4).

Summary

2,4-D and picloram exhibit low toxicity to all fresh water and marine algae examined even at rates approaching the maximum solubility of the herbicides in water. Use of formulated materials (especially esters) increases toxicity. Yet, these herbicides present a low potential hazard to aquatic ecosystems from normal agricultural or industrial use practices as might be encountered for vegetation control along Indiana roadsides.

Acknowledgements

This portion of the report is from a study with J. H. Elder and C. A. Lembi. Mr. Elder was a graduate student and received OWRR support. Dr. C. A. Lembi was a post-doctoral associate whose tenure was sponsored through a grant from the National Science Foundation.

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TOXICITY OF DICAMBA (BANVEL) AND M3766 (PICLORAM + 2,4-D) TO ALGAE AND FISH

Introduction

Previous studies from another project (1) demonstrated that approximately 25% of the picloram in a herbicide mixture could be replaced with 2,4-D without any decrease in treatment effectiveness. Based on these findings, a special formulation of picloram (Tordon) + 2,4-D was developed in cooperation with the Dow Chemical Company, Midland, Michigan. The mixture is a combination of three parts of picloram and one part 2,4-D (2 lb/gal. total herbicide) plus an agent to control drift. The formulation, designated M-3766, consists of amine salts of the herbicide determined from the preceeding portions of this report to be non-toxic to fish and algae in laboratory tests. The formulation is aimed at environmental safety especially to aquatic chain organisms. However, it was necessary to submit the formulation to a series of tests similar to those for the individual components of the mixture to guard against the possibility of some type of interaction leading to toxicity.

A second material, dicamba (Trade name Banvel) was also included in these tests. This material has been reported to be non-toxic to fish and other aquatic organisms and might possibly prove useful in the control of brush along county roads. This material was advertised as defoliating more slowly so that conspicuous "brown-out" might be avoided.

Materials and Methods

Dicamba (2-methoxy-3,6-dichlorobenzoic acid, technical grade) and M-3766 (a 3:1 mixture of picloram (4-amino-3,5,6-trichloropicolinic acid and 2,4-D (2,4-dichlorophenoxyacetic acid)) were added to the growth medium of various algal cultures at 2.5, 25, and 250 ppm concentrations. The various genera of algae examined are summarized in Table 9. All were fresh water genera and were grown on an optimal medium in a greenhouse. Cells were examined for a period of 4 weeks with the light microscope to determine degree of injury.

For experiments with fish, green sunfish (Lepomis cyanellus) were maintained in tap water with continuous aeration and on an artificial diet (Master Mix Trout Food). The fish were exposed to 2.5, 25 and 250 ppm levels of either dicamba or M3766.for 1 week at 22^{o} C. Fish mortality was recorded and at the end of the study, fish were examined for tissue abnormalities. The livers were excised, weighed and sampled for histological examination.

Results and Discussion

The growth characteristics of the various algal genera tested are given in Table 10. With most species, a definite sequence of recognizable symptoms were associated with herbicide injury. For the motile forms, the colony would first become immobilized. Next, the colony would break up, but the cells would still be green. Finally, the cells would lose chlorophyll and die. With non-motile colonies, the colony would break up into still green cells followed by loss of chlorophyll and death. For filaments, the filaments would first break up into shorter segments with a few dead cells. Finally, all

Table 9. Algal genera tested

Division Chlorophyta

Order Cladophorales

Pithophora

Order Oedogoniales

Oedogonium

Order Volvocales

Carteria

Gonium

Eudorina

Order Chlorococcales

Pediastrum

Ankistrodesmus

Order Zygnematales

Closterium

Division Chrysophyta

Tribonema

Division Cyanophyta

Oscillatoria

Nostoc

Anabaena

Table 10. Growth characteristics of algal genera tested

Pithophora- branched filament.

Oedogonium- unbranched filament.

Carteria- unicellular motile.

Gonium- motile colony.

Eudorina- motile colony.

Pediastrum- non-motile colony.

Ankistrodesmus- filament.

Closterium - unicellular, non-motile

Tribonema- unbranched filaments.

Oscillatoria- filaments that glide, no sheath surrounding cells.

Nostoc- unbranched filaments, sheath surrounding cells.

Anabaena- unbranched filaments, no sheath.

the cells in the shortened filaments would die.

M-3766 at 250 ppm was toxic to most of the algae (Table 11). This may be due to the impurity 2-(3,4,5,6-tetrachloro-2-pyridyl)-guanidine and in this respect the mixture resembles technical grade picloram (2, 3, 4). However, Pithosphora, Nostoc, and Anabaena were filaments that were not injured by M-3766 even at 250 ppm. These species are notoriously resistent to herbicides. Carteria was unusual in that it was a motile form not injured by M-3766 even at 250 ppm. As with technical picloram, except for Carteria, motile forms were more sensitive to M-3766 than non-motile forms. M-3766 at 25 ppm or 2.5 ppm was not toxic to any of the algal genera tested.

Dicamba (Banvel) showed no significant toxicity to any of the algae tested at any concentration. Eudorina and Pediastrum showed slight indications of sensitivity to dicamba at 250 ppm but were not killed.

As with technical picloram, fish were rapidly immobilized by M-3766 at 250 ppm. 100% mortality occurred within a few hours. At 25 ppm M-3766, the fish behaved sluggishly for a time but no mortality was observed. M-3766 at 2.5 ppm and all levels of dicamba, including 250 ppm the highest concentration tested, had no effect on the fish. In these experiments, we observed no significant effect on liver enlargement from any of the treatments (Table 12) with dicamba but enlargement was indicated at the 250 ppm level of M-3766.

Table 11. Effect of M-3766 or dicamba on survival of various algal genera*.

	Dicamba			M-3766		
Organism	2.5 ppm	25 ppm	250 ppm	2.5 ppm	25 ppm	250 ppm
Pithophora	-	-	-	-	-	-
Oedogonium	-	-	-	-	-	+
Carteria	-	-	-	-	-	-
Gonium	-	-	-	-	-	+
Eudorina	-	-	±	-	-	+
Pediastrum	-	-	±	-	-	+
Ankistrodesmus	-	-	-	-	-	+
Closterium	-	-	-	-	-	+
Tribonema	-	-	-	-	-	+
Oscillatoria	-	-	-	-	-	+
Nostoc	-	-	-	-	-	-
Anabena	-	-	-	-	-	-

* + = Concentration of herbicide cause injury or death of cells.

- = Concentration of herbicide was without effect.

Table 12. Effect of dicamba and M-3766 on liver weight of green sunfish (Lepomis cyanellus)

Herbicide	Conc.	Detn.	Fish Wt.	Liver Wt.	Liver/Fish	Ave.
Dicamba	2.5 ppm	1	7.84g	0.09g	0.0114	0.0135
		2	17.16	0.27	0.0157	
	25 ppm	1	8.27	0.09	0.0108	0.0105
		2	13.61	0.14	0.0102	
	250 ppm	1	11.68	0.16	0.0136	0.0120
		2	9.50	0.10	0.0105	
Control	-	1	13.86	0.13	0.0093	0.0110
		2	8.53	0.11	0.0128	
M-3766	2.5 ppm	1	16.32	0.21	0.0128	0.0112
		2	8.32	0.08	0.0096	
	25 ppm	1	13.67	0.18	0.0131	0.0118
		2	12.17	0.13	0.0106	
	250 ppm	1	29.12	0.34	0.0116	0.0137
		2	15.10	0.24	0.0158	

Summary

The toxicity of M-3766 is not substantially different from that of technical picloram alone to either green sunfish or fresh water algal genera. The alga Carteria was shown to be a resistant motile form. Dicamba (Banvel) was virtually nontoxic.

Acknowledgements

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THE METABOLISM OF ^{14}C-PICLORAM BY THE PICLORAM-RESISTANT MOTILE ALGA, CARTERIA SP.

Introduction

In the preceding section of this report, we demonstrated that, of the motile algal genera tested, only Carteria was resistant to technical grade picloram containing the toxic impurity 2-(3,4,5,6-tetrachloro-2-pyridyl)-guanidine. Carteria retained motility at concentrations of 250 ppm of technical picloram while other motile species did not. To determine if Carteria was able to metabolize picloram and thereby escape its toxic action, experiments were carried out with ^{14}C-labeled picloram using this organism.

Materials and Methods

^{14}C-picloram (48.5 mc/mg) was obtained through the courtesy of the Dow Chemical Company, Midland, Michigan. The picloram was dissolved in ethanol at a concentration of 2 mg/ml and diluted 1:100 with growth medium containing the cells to be tested. The final concentration of picloram was 0.02 mg/ml. The cells were grown in continuous light (about 150 ft.-c.) in an isotope hood and 0.1 ml samples were withdrawn at 1 day intervals for a period of 14 days. All measurements were referred to a control flask containing radioactive picloram and growth medium but no cells. The experiment was repeated 3 times comparing fresh inoculum and an inoculum consisting of conditioned cells which had been grown for several weeks in the presence of 250 ppm M-3766.

For determination of radioactivity, samples were mixed with 10 mls of Bray's solution and placed in a liquid scintillation counter.

Results and Discussion

The ratio of radioactivity of the flasks containing cells to that of the flask containing no cells declined during the first 24 hours of incubation to a level of about 92%, i.e. 8% of the radioactivity was metabolized, and then remained constant for the duration of the experiment.

In the third experiment of this series, the flasks were attached to a CO_2 trap containing 250 ml 2.5 N potassium hydroxide on the inlet side and a CO_2 trap containing 10 ml of 1.0 M ethanolamine on the discharge side. These studies indicated an initial rapid burst of radioactive CO_2 but no additional radioactive CO_2 was released during the course of the experiment.

The findings establish conclusively that Carteria is unable to carry out any significant metabolism of picloram. However, the possibility is raised that the organism is able to metabolize the toxic impurities in technical picloram and thereby escape the toxic action of this material.

Summary

The motile alga Carteria does not metabolize picloram. The results suggest that it may metabolize the toxic impurities contained in technical picloram as the basis for its resistance to this material.

Acknowledgements

These studies were conducted by Mr. Brian Ritenour. The sample of radioactive picloram was supplied by the Dow Chemical Company, Midland, Michigan.

DEVELOPMENT OF A BINDING ASSAY FOR HERBICIDE RECEPTORS USING INDOLE-3-ACETIC ACID AS A MODEL

Introduction

A major objective of the proposed research was to develop a new approach to seek evidence (or the lack of it) for specific herbicide receptors or binding sites in fish, algae, and mammals. Preliminary studies had suggested that 2,4-D is remarkably specific it its mode of action (6, 7, 8, 10,11, 17, 28). It appears to be toxic only to vascular plants (higher plants) and not to algae or animals. The reason for this specificity is not known but we suspect that it is related to the presence of specific "receptor" proteins located on the surface membranes of individual cells. We developed procedures to isolate the surface membranes of plant and animal cells (12, 13, 20, 28) and began to develop techniques to locate the "receptors" on the membranes. There was reason to believe that only higher plants contained the receptors and that they were absent from animals and all forms of microscopic life. Could this be demonstrated, it would constitute a form of proof that not only are the herbicides in question non-toxic to all forms of life other than the target organisms (weeds, brush, and other higher plants) but that other forms of life (including man, domestic animals, and aquatic organisms) lack the biochemical machinery to respond to the herbicide. It is this type of information that will eventually be required to settle the problem of herbicide safety to man and his environment.

The development of methods to isolate and identify plasma membranes from plant cells (6, 12, 13, 20, 28) makes possible direct assay of auxin binding sites at the plasma membrane. Binding of the auxin antagonist, N-1-naphthylphthalamic acid (NPA) to cell fractions has been shown to correlate directly with the plasma membrane content of fractions from corn coleoptiles (13). Subsequently, Hertel and coworkers (7) found the binding of the auxins indole-3-acetic acid (IAA) and naphthalene-3-acetic acid (NAA) to be associated with membrane fragments in cell homogenates of maize coleoptiles. In this study, we examined the binding of the auxin IAA to defined cell fractions and show that, in soybean hypocotyls, the highest specific activity of auxin binding is found in fractions rich in a "heavy" component of the surface or plasma membrane.

Materials and methods

Soybeans (Glycine max L., cv. Wayne) were grown in moist vermiculite in the dark for 4 days. Corn (Zea mays L, cv. WF-9 X M-14) seedlings were grown by Method II of Morre et al. (18). Whole hypocotyls with the cotyledons and apices removed or coleoptiles (with leaf rolls removed) were homogenized in the coconut milk medium described previously (13) using a Polytron 20ST homogenizer (Kinematica, Lucerne, Switzerland) at approximately 9,000 rpm for 2 min. After squeezing through a single layer of Miracloth (Chicopee Mill, N. Y.), the homogenate was centrifuged at 20,000 x g (Sorvall, HB4 rotor) for 12 min. The supernatant was layered onto a discontinuous gradient consisting of 0.65, 0.8, 1.0, 1.2, and 1.3 M sucrose in coconut milk medium, and centrifuged for 1 h at 80,000 X g (Spinco, SW 27.1 rotor).

Thefractionation achieved is diagrammed in Figure 12. Fractions were removed from the gradients, diluted, pelletted at 80,000 X g for 45 min and resuspended in a small volume of 0.25 M sucrose adjusted to pH 7.4 with Tris (tris-(hydroxymethyl)-1-aminomethane) base. The distribution of cell components in the various gradient fractions was determined by quantitative electron microscope morphometry according to the procedure of Lembi (13).

Auxin Binding Assay

Two different suspension media were used. The Tris medium contained 25 mM Tris, 0.5 mM $CaCl_2$, 4 mM mercaptoethanol and 0.3 M sucrose, adjusted to pH 6 with 0.2 M KH_2PO_4. The Tris-MES medium contained 80 mM MES (2-(N-morpholino)-ethanesulfonic acid), 14 mM mercaptoethanol, and 0.63 M sucrose (20%) adjusted to pH 6 with acetic acid (7). Both suspension media gave similar results. IAA was added as an acetonitrile solution.

Assays were at 4^{o} in two series. For series A: To a 5.4 ml cellulose nitrate tube were added 0.1 ml acetonitrile, 0.1 ml acetonitrile containing 5 X 10^{-6} M IAA-2-^{14}C (Radiochemical Centre, Amersham, England), 0.2 ml cell fraction and 4.6 ml of either Tris or Tris-MES medium. Series B was identical except for the addition of 0.1 ml 5 X 10^{-3} M unlabeled IAA in acetonitrile in place of the acetonitrile. The contents of the tubes were mixed by inversion, and centrifuged immediately and in parallel at 80,0000 X g (Spinco SW 50.1) for 20 min. The supernatants were decanted and the tubes were left in an inverted position to drain for about 1 h. The bottom of each tube was then cut off approximately 1 mm above the

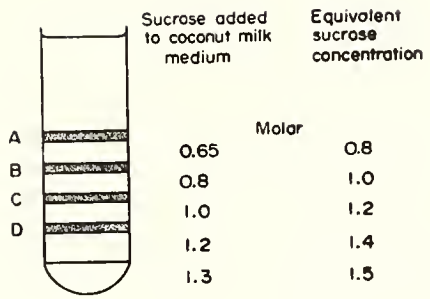

Figure 12. Summary of procedure for sucrose-coconut milk gradient centrifugation.

top of the pellet, and transferred to a counting vial. Water (0.5 ml) was added and the pellets were resuspended by sonication (Branson Sonifier, "Special micro tip"). Material adhering to the tip was washed into the vial with 0.5 ml water. Bray's solution (15 ml) was added and radioactivity determined using a Packard liquid scintillation spectrometer. Radioactivity specifically bound to cell fractions was determined as the difference between the sample in series A (total radioactivity) and the sample in series B (radioactivity not specifically bound). Proteins were determined from separate aliquots by the method of Lowry et al.(15), and mitochondrial activity was monitored by assay of succinate dehydrogenase with INT (iodonitrotetrazolium) as electron acceptor (22).

Results

Table 13 shows the distribution of auxin binding activity among crude cell fractions separated by differential centrifugation of soybean hypocotyl and corn coleoptile homogenates. With both tissues, binding of indoleacetic acid (IAA) was found in the pelleted fractions, with the 2,000-20,000 X g pellet having the greatest specific activity. To determine what cell component(s) were binding the IAA, the 2,000-20,000 X g pellets for each tissue were fractionated into mitochondria-rich and mitochondria-poor fractions by centrifugation through 1.25 M sucrose. The identity of each of the fractions was verified by microscopy. Table 14 shows that the auxin binding activity was retained on the 1.25 M sucrose layer while mitochondria were predominantly in the pellet as indicated by succinate-INT-reductase. Binding was associated with the

Table 13. Binding of indoleacetic acid by centrifugally-separated cell fractions of etiolated maize and soybean

Cell fraction	Series	Maize		Soybeans	
		Cpm	Cpm/mg protein	Cpm	Cpm/mg protein
2,000-100,000 X g pellet	A	646		725	
	B	591		680	
	A-B	55	21	45	33
20,000-100,000 X g pellet	A	360		699	
	B	340		673	
	A-B	20	14	26	23
20,000 X g supernatant	A	360		623	
	B	342		609	
	A-B	18	22	14	11
2,000-20,000 X g pellet	A	445		493	
	B	429		469	
	A-B	16	54	24	43

Table 14. A comparison of subfractions of the 2,000-20,000 x g pellets for auxin binding and mitochondrial activity

Subfraction	Auxin (IAA)-binding (Cpm/mg protein)		Succinate-INT-reductase (μMoles/h/mg protein)		% Plasma Memb (by morphometry)
	Maize	Soybeans	Maize	Soybeans	Soybeans
1.25 M sucrose layer	61	76	0.80	1.25	50 ± 6
Pellet	23	5	2.80	3.27	3 ± 1

predominantly non-mitochondrial portions of the fractions. Electron microscopy of the auxin-binding fractions revealed many profiles identified as plasma membrane by the cytochemical procedure of Roland et al. (26).

Sucrose-gradient fraction of the 20,000 X g supernatant gave highest auxin binding activity in fraction D (Table 15). Fraction D consists almost exclusively of mitochondria and plasma membrane while the greatest concentration of plasma membranes was in fraction C as snown in Table 16. To re-examine the possibility of auxin binding to mitochondria versus binding to plasma membranes, fraction D was resolved into mitochondrion-rich and plasma membrane-rich fractions. It was noted that when fraction D was pelleted, there was a visible layering of the pellet. When the two layers were resuspended separately and assayed for auxin binding and succinate dehydrogenase, the auxin binding was associated with the plasma membrane rather than with the mitochondria (Table 17). Auxin binding correlated approximately with plasma membrane content within an individual subfractionation experiment but not among different experiments. An experiment with the same rationale as that of Table 17 was to vary the time of centrifugation at 20,000 X g prior to placing the supernatant on the sucrose gradient. A 12 min centrifugation at 20,000 X g prior to the sucrose gradient step removed the bulk of the mitochondria and left fraction D with a higher specific activity of auxin binding than did a 5 min pre-centrifugation which removed mostly nuclei and cell fragments but fewer mitochondria (Table 18). Comparisons were made of fractions from etiolated hypocotyls with those

Table 15. Auxin-binding activities of cell fractions of etiolated soybeans separated by sucrose gradient centrifugation

Cell fraction	Series	Total cpm/determination				Cpm/mg
		I	II	III	Ave	protein
2,000-20,000 X g pellet	A	846	1611	1828	1428	
	B	800	1542	1687	1343	
	A-B	46	69	141	85	133
20,000 X g supernatant:						
A. 0.6/0.8 M sucrose	A	1689	1640	1520	1616	
	B	1569	1601	1516	1562	
	A-B	120	39	4	54	61
B. 0.8/1.0 M sucrose	A	1587	1616	1367	1523	
	B	1505	1603	1340	1482	
	A-B	82	13	27	41	49
C. 1.0/1.2 M sucrose	A	1666	1690	1250	1535	
	B	1621	1654	1198	1490	
	A-B	45	37	52	45	62
D. 1.2/1.4 M sucrose	A	1102	1035	1241	1126	
	B	1010	1010	1174	1061	
	A-B	92	25	67	61	93

Table 16. Distribution of cell components among cell fractions from soybean determined by quantitative morphometry from electron micrographs of the four fractions (A-D) of Table 15.

Cell component	% of total membrane profiles			
	A	B	C	D
Plasma membranes[a]	12 ± 2	26 ± 7	68 ± 10	43 ± 12
Dictyosomes	35 ± 13	34 ± 6	6 ± 3	Trace
Mitochondria	Trace	1 ± 0	12 ± 5	55 ± 7
Endoplasmic reticulum, microbodies and nuclei	3 ± 3	1 ± 1	Trace	Trace
Tonoplast, plastid fragments	50 ± 12	38 ± 6	14 ± 4	2 ± 2

[a]Identified on the basis of the PTA-chromic acid staining. All other cell components were determined from sections stained with lead citrate.

Table 17. Comparison of subfractions of the 1.2/1.4 M sucrose (D) fraction from soybeans for auxin-binding and content of mitochondria and plasma membrane.

Membrane subfraction	Specific activity		Plasma membrane
	Auxin(IAA)-binding (Cpm/mg protein)	Succinate-INT-reductase (µMoles/h/mg protein)	%
2,000 - 20,000 X g pellet[a]	92	2.69	15 ± 5
D_{top} (Plasma membrane-rich)	203	0.83	55 ± 10
D_{bottom} (Mitochondria-rich)	3	3.11	2 ± 2

[a]Crude mitochondria for comparison.

Table 18. Comparison of subfractions of the 2,000 - 20,000 X g pellet and the 1.2/1.4 M sucrose (D) fraction of soybeans prepared by differential centrifugation for auxin binding and mitochondrial activity

Fraction	Centrifugation	Specific activity	
	(Time at 20,000 X g)	Auxin(IAA)-binding (Cpm/mg protein)	Succinate-INT-reductase (μMoles/h/mg protein)
2,000 - 20,000 x g pellet	5 min	27	2.24
	12 min	25	1.64
1.2/1.4 M sucrose (D fraction)	5 min	31	2.16
	12 min	135	1.62

from hypocotyls exposed to white light for 16 h prior to homogenization. In these experiments, there was an overall depression of auxin binding capacity of the fractions from light-grown plants and also a shift in the binding capacity toward the lighter C fraction. The plasma membrane content of the fractions was unaffected (less than 10%) by light.

Discussion

The concept of an auxin-membrane interaction is not new (19). Such implications were implicit in the structure-function correlations of Bungenberg de Jong and his colleagues (1), those of other auxin pioneers (29, 30) and form work with mammalian membranes (2, 3, 4, 5, 9). RNA and protein synthesis are required for sustained auxin growth (10, 11) but when auxins are supplied under conditions which ensure rapid entry into the cell, auxin growth is initiated without appreciable lag (21, 24). The existence of rapid growth and other responses to auxin (cf. 27) gives support to a concept of auxin action where the hormone acts near its site of initial interaction with the cell, i.e. at or near the cell surface.

Progress in demonstrating and characterizing auxin-plasma membrane interactions has been facilitated by methods for isolating and indentifying plasma membrane fragments from plant cells. The methods employed for the auxin studies reported here utilize homogenization in a medium prepared in coconut milk and purification on sucrose gradients prepared in coconut milk, a membrane-free source of protective cytoplasmic constituents. The degree of auxin responsiveness of the membranes may be related to the quality of functional preservation attendant with

isolation in the coconut milk medium. To demonstrate the presence of auxin receptors at the plasma membrane, the procedure of Hertel and Thomson (13) was employed. The test measures reversible binding and is auxin-specific. Benzoic acid does not compete. The growth active D-2,4-dichlorophenoxyisopropionic acid inhibits ^{14}C-IAA binding more than the less active L-isomer.

Our findings show that the most active auxin-binding fractions of etiolated maize coleoptiles and of soybean hypocotyls are those enriched in plasma membranes. Yet, different plasma membrane fractions vary in their capacity to bind auxins. Those fractions which contain heavy plasma membrane fragments, usually contaminated with mitochondrial membranes, have the greatest activity. When resolved into mitochondrion- and plasma membrane-rich subfractions, the auxin binding activity is purified with the plasma membrane-rich subfraction.

The auxin binding activities are low but representative of those expected from estimates from physiologically active intra-cellular auxin concentrations (Hertel *et al.*, private communication). Thus, one is forced to conclude that if any membrane fraction binds auxin, it is a fraction containing plasma membranes. We find no enrichment of auxin binding activity in purified mitochondrial fractions and are able to account for the entire composition of the D fraction on the basis of mitochondria and heavy plasma membranes. Thus, notwithstanding the possibility of some mitochondrial subfraction or mitochondrion-like plastid fraction being responsible for auxin binding, we conclude that plasma membranes bind auxin.

Experimental work on auxin transport had likewise led to the concept of an involvement of attachment to the plasma membrane (8, 15). Hertel et al. (7) have suggested that the transport site of attachment might be the same as the growth regulating site of attachment. To what extent the auxin binding reported here is related to auxin transport is unknown. Hertel et al. (7) compared the ability of various auxins to be transported polarly with their ability to cause rapid cell elongation and found a close correlation.

Why does fraction C, a fraction consistently enriched in plasma membranes (Table 16) not bind auxin to the same extent as fraction D? One explanation is that there is a heterogeneity of plasma membranes and that separation of at least two types of plasma membranes occur in our fractionation scheme. This agrees with the findings of Hertel et al. in which they report two classes of membranes both of which bind the auxin transport antagonist NPA but only one of which, the denser or heavier fraction, binds auxin. There is also a tendency for the PTA-chromic acid procedure of Roland et al. (26) to give a more intense stain with the "heavy" plasma membrane fraction and the membranes are slightly thicker in the D fraction compared to the C fraction. Thus etiolated soybean hypocotyls contain both light and heavy plasma membrane fractions with heavy plasma membranes being the most effective in binding auxins.

Light-grown hypocotyls yielded light and heavy plasma membrane fractions but overall auxin-binding capacity was depressed relative to corresponding fractions of etiolated tissues. With light-grown hypocotyls, the light and heavy plasma membrane fractions were more nearly equal in their capacity to bind auxins. The reduction in auxin-binding capacity

of plasma membranes attendent with exposure of the hypocotyls to light correlates with the well-known lack of auxin responsiveness in light-grown relative to etiolated tissues (14).

One important question which emerges concerns the nature and origins of the different species of plasma membrane. Are the different types derived from different cell types or can the plasma membrane of a single cell give rise to different species of plasma membrane vesicles, one showing a specific interaction with auxin and the other not?

Summary

The binding of auxin, indole-3-acetic acid, to defined cell fractions from corn coleoptiles and soybean hypocotyls was studied. The results show the highest specific activity of auxin binding in fractions rich in a "heavy" component of the plasma membrane. The auxin binding assay developed in this study provides a means to seek evidence for specific herbicide receptors or binding sites in other organisms including fish, algae, and mammals and was used for that purpose as summarized in the next section of this report.

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APPLICATION OF BINDING ASSAY FOR HERBICIDE RECEPTORS TO 2,4-D SITES

Introduction

To yield definitive answers on the safety of herbicides, the approach of measuring specific binding sites on the cellular membranes was utilized. The theoretical basis and background information leading to this approach is summarized in the preceding section of this report.

Materials and Methods

The basic approach was to prepare plasma membranes and other cell fractions following the procedures just described. Using the specific herbicide binding assay developed for indoleacetic acid, quantitative data on the distribution of 2,4-D receptors was sought. The comparisons included green sunfish (Lepomis cyanellus), mixed unicellular green algae, Spirogyra, soybeans, duckweed (Lemna minor) and rat liver. The rat liver fractions were prepared as detailed by Morré (1).

2,4-D-^{14}C (250 mC/1 = 250 μC/ml) was dissolved in the ratio of 0.1 ml radioactive 2,4-D per 30 ml acetonitrile to give a final level of radioactivity of approximately 80,000 cpm/ml. Unlabelled 2,4-D was prepared at a final concentration of 5×10^{-2} M in acetonitrile and added to a portion of the radioactive material for the B solution. The remainder of the assay was as just described for indoleacetic acid.

The unicellular green algal genera tested included the following: Navicula, Euglena, Trachelomonas, Desmidium, and Cosmarium. Euglena and Trachelomonas were in most abundance.

Results and Discussion

Plasma membrane fractions from soybeans bound 2,4-D with an efficiency about 3-times that of endoplasmic reticulum and mitochondrial fractions but less efficiently than they bound the natural auxin indoleacetic acid. This difference was expected and does not detract from the validity of the approach although any quantitative interpretation in terms of numbers of binding sites is severly curtailed.

Plasma membranes or mitochondria from rat liver did not show specific binding of 2,4-D within the detection limits of the assay nor did membrane fractions from fish, unicellular algae or duckweed (Table 19). Spirogyra, a 2,4-D resistant filamentous green algae, did show strong indications of binding the 2,4-D. Endoplasmic reticulum fractions from rat liver were also found to bind 2,4-D and 2,4-D binding of endoplasmic reticulum membranes of plants was greatly increased if ribosomes were present on the membranes.

Considering the low affinity of binding or low concentration of sites for 2,4-D binding of plant membranes and possible complications arising from 2,4-D binding to ribosomes, it is doubtful whether or not the data of Table 19 do much to dispell fears concerning herbicide toxicity to mammals and algae. However, the approach is working and with additional refinements may yet provide definitive answers.

Table 19. Herbicide-binding activities of cell fractions of etiolated soybeans and algal, fish and mammalian (rat liver) sources.

	cpm ^{14}C-2,4-D bound/mg protein			
Cell fraction or source	Expt. I	Expt. II	Expt. III	Ave.
Higher Plant				
Soybean plasma memb.	44.0	34.4	17.9	32.1
Soybean endoplasmic reticulum	16.4	0.0	3.5	10.0
Soybean mitochondria	12.2	9.0	19.7	13.6
Mammalian (Rat Liver)				
Plasma membrane	14.8			
Mitochondria	8.2			
Fish (muscle membranes)	14.9			
Algae Membranes				
Spirogyra	39.2			
Mixed Unicellular	13.8			
Higher Plant (Aquatic)				
Duckweed	16.4			

Summary

The binding of the herbicide 2,4-D to defined cell fractions from higher plants, unicellular algae, filamentous algae, a primitive aquatic higher plant (duckweed), fish and rat liver. Only the fractions containing the plasma membrane from higher plants and the fraction from Spirogyra (filamentous green alga) showed evidence of specific 2,4-D binding. Plasma membranes from rat liver and fractions from fish muscle and unicellular green algae or duckweed did not. The low specific activity of binding and possible binding of 2,4-D to ribosomes complicates the utility of the assay in its present form. More sensitive assay procedures along similar lines are currently being sought.

Acknowledgements

These experiments were conducted by Ms. Kristine Hess, an undergraduate student in Biological Sciences at Purdue University with the assistance of Mr. Brian Ritenour.

Reference

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DEVELOPMENT OF AN ELECTRON MICROSCOPE ASSAY FOR HERBICIDE BINDING

Structural changes in plasma membranes of etiolated soybean hypocotyls were induced by indole-3-acetic acid, picloram, and 2,4-D and monitored by electron microscopy. Isolated and in situ membranes were stained by a phosphotungstate-chromate procedure to identify and accentuate plasma membrane. Measurements were on micrographs obtained with an electron optical system calibrated and corrected for reproducible and accurate size measurements. In preparations treated for 20 min with 1 μM IAA, picloram or 2,4-D, the plasma membranes were approximately 0.85 as thick as controls. Multiple cycles of IAA-control treatment yielded membranes with dimensions that reflected the last treatment of the series. The response is independent of pH but dependent on temperature. The dose-response relationships of IAA and the other herbicides for both growth and the induced membrane transformation were similar. The findings show a direct conformational response of plasma membranes to growth regulating agents that lends itself to ready quantitation.

These findings are being readied for publication and will be published in detail. I anticipate that the electron microscope assay will overcome the shortcomings of the 2,4-D binding assay and provide the necessary mode-of-action information required to evaluate the safety of herbicides from this standpoint.

PERSISTENCE AND MOVEMENT OF PICLORAM IN WATER AND SOIL

I. DEVELOPMENT OF BIOASSAY PROCEDURES

II. EVALUATION OF POTENTIAL INJURY TO NONTARGET VEGETATION

Introduction

Determination of the movement and accumulation of picloram in water, soil and aquatic biota depends on the development of suitable bioassay procedures that are sensitive and specific for picloram. Picloram (Tordon Herbicide) is a potent herbicide providing control of a broad spectrum of plant species and was developed primarily for control of brush and deep-rooted herbaceous species in non-crop areas. Problems relating to the persistence of this material are summarized in the references (1-3).

Analyses for picloram in plant and soil residues as well as in runoff water and streams are essential to its continued use and testing. Quantitative information on the rate of entry and accumulation of picloram and related herbicides into fresh water streams from existing and projected use practices is very limited. Information is also needed to permit evaluation of the effects of picloram on fresh water ecosystems. To what extent biological magnification within the food chain might accentuate picloram toxicity to aquatic organisms must also be verified.

Materials and Methods

During the course of physiological studies, it was discovered that picloram was capable of eliciting a unique plant response--that of rapid elongation of epicotyls of intact, light-grown seedlings (4, 5). In preliminary studies, no other compounds have been found which are capable of eliciting this type of response including other herbicides of the auxin type such as 2,4-D and 2,4,5-T. Under this portion

of the project, the epicotyl growth system was used as a bioassay procedure for quantitative determination of picloram in runoff water, soil or soil and plant extracts either in the presence or absence of other herbicides (e.g. M-3766) or even nonspecific toxins. The assay is sensitive with ready detection in the picogram range.
The procedure involved developing controlled conditions for carrying out the assay (precondition of the biological material, temperature, light intensity, pH, solvents) to maximize sensitivity and reproducibility. Standard curves were obtained in the presence and absence of potential interfering substances, and from known quantities of the herbicide mixed with soil or plant extracts.

A second assay procedure involved the use of potato tubers. Potato (Solanum tuberosa, cv. Kennebec) tubers were cut giving about 2 buds/explant and planted directly into soil suspected of containing picloram residues. The presence of picloram elicits a very characteristic response (an etiolated appearance) in the plants (Fig. 13) compared to normal potatoes (Fig. 14). A third assay procedure is to plant soybean (Glycine max, cv. Hawkeye) seeds directly into the treated soil(Fig.15). A characteristic plant response is produced in the picloram-treated soybeans in which the leaf tips appear long and pointed (Fig. 16). The limit of detection for either the potato or bean assay has not been determined and these two techniques were utilized primarily as qualitative assays.

A final assay procedure developed under this project utilized intact seedlings of American Basswood (Tilia americana). In this assay seedlings were removed from the soil (about 1 ft. high and

Figure 13. Potato plants frown from tuber explants in soil sprayed 1 year previous with 2 lb/A of M-3766, a rate equivalent to 1½ lb picloram/acre. Photographed June 1, 1974.

Figure 14. Control potato plants of the same age as those of Figure 13.

Figure 15. Soybean seedlings planted in a test plot to determine the presence of picloram.

Figure 16. Soybean plants showing evidence of chronic picloram toxicity. The long and pointed leaf tips are characteristic of picloram injury. These plants are from a field adjacent to 4 lb/A of M-3766 the summer following a fall application. The plants of Fig. 15 were from seeds planted Aug. 23 with the photograph taken Sept. 12 one year following application of 1 lb/A of M-3766. These plants appear normal. The M-3766 was applied in early October 1972.

with 6-8 fully expanded leaves), the roots were washed free of soil, and the seedlings were placed in 500 ml containers with approximately 250 ml of the solution to be tested. Within about 4 days after introduction, the seedlings showed evidence of petiole twisting and a characteristic yellowing of the leaves. Within one week, most of the seedlings watered with picloram-containing solutions will have lost most or all of their leaves. The tests were discontinued after 2 weeks since no additional symptom development could be observed beyond that time. The lower limit of detection is 10^{-9} M picloram (ca. 0.1 parts per billion) making this bioassay one of the most sensitive known for herbicides (Table 20) and certainly the most sensitive ever developed for picloram.

Results and Discussion

In early October 1972, mid-September 1973 and late May 1973 "total environment" test plots were established in Tippecanoe and White Counties, Indiana,using truck-mounted equipment. The picloram + 2,4-D combination M-3766 was applied at rates of 0.5 to 4 lb/A of total herbicide with 1-2 lb/A being a practical rate.

Included in the tests were ornamental plantings, hardwood forest, conifer stands, an orchard, a vegetable garden, soybean, corn, small grain and legume fields, permanent pasture, and a flowing stream paralleling the sprayed area. At no time were these areas directly oversprayed. However, the areas adjacent to them were oversprayed directly to determine how well the treatment was contained within the target area.

Table 20. Characteristics of the Tilia americana bioassay for picloram.

Picloram Conc. (M)	Yellowing of Leaves/ Petiole Twisting			Defoliation/Death		
	4 days	7 days	14 days	4 days	7 days	14 days
10^{-6} (250 ppb)	+				+	+
10^{-7} (25 ppb)	+				+	+
10^{-8} (2.5 ppb)	+	+				+
10^{-9} (0.25 ppb)		+	+			$\pm$
10^{-10} (0.025 ppb)			-			-

Testing of water samples using the mung bean bioassay (sensitive to about 1 ppb) revealed insignificant quantities of herbicide (less than 0.1 ppm) entering the water from drift at the time of spraying and no detectable herbicide from leaching or runoff including samples collected during the first heavy rain following spraying. These tests were carried out during the six month period following the application in October of 1972. Tests of soil samples also during this period using the mung bean assay showed that the herbicide remained on the target area and was biodegraded at the expected rate. By the following fall, soybeans could be grown in the test plots sprayed directly with 1 lb/A of M-3766 (3/4 lb/A of picloram)(Fig. 15) but not in plots sprayed directly with the 2 lb/A rate. At the 2 lb/A rate, residues could not be detected using either the soybean or potato assay two years following treatment. Similar findings have been obtained to date with the test begun in mid-September 1973.

In the 1972 environmental test, we noted no significant injury to ornamental, orchard, or hardwood species not directly oversprayed with the chemical. Figure 17 shows the appearance of new tree growth from small trees (less than 8 ft. in height) growing within 3 feet of the edge of the spray pattern. These twigs were collected and photographed April 23, 1973. The area adjacent to where the trees were growing had received an application of 2 lb/A M-3766.

As mentioned above, cropland directly oversprayed with 1 lb/A of M-3766 in the fall will produce some injury to soybeans the following spring. Some injury to soybeans has been consistently noted in the outside row immediately adjacent to plots treated with 1 lb/A M-3766 and

Figure 17. New tree growth from small trees less than 8 ft. in height collected from specimens growing within 3 feet of the edge of the spray pattern. The area adjacent to these trees received an application of 2 lb/A of M-3766 (1½ lb/A of picloram + ½ lb/A of 2,4-D) in early October 1972. The twigs were collected and photographed April 23, 1973.

Species are shown with a control branch on the left and a branch from the treated area on the right.

From left to right, top row: Apple and Basswood. The treated basswood branches showed more anthocyanin in the buds. Bottom row: Red-stemmed Dogwood (some strap leaves in treated); Ironwood (treated essentially normal); and Black Cherry (some leaf curling in treated). These species were representative of the 30+ species examined in the test.

and especially with 2 lb/A of M-3766. No injury was detected with ½ lb/A. One year following application, soil residues have dropped below the phytotoxicity level to soybeans at the ½ and 1 lb/A rate even on the treated areas but continue to persist on the treated area at the 2 lb/A rate. No evidence of injury from drift or overspraying was noted for corn, legumes or grass.

In three years of testing, only one instance of serious injury to non-target vegetation was noted from lateral movement of the herbicide either through the soil, by erosion, or by runoff water. The injury occurred in a soybean field adjacent to a test area treated with 4 lb/A M-3766. The area received water draining from about 1 mile of test plot and in the low spot in the field where the water stood, serious picloram injury was noted (Figure 18). Even here, the plants survived and the yield reduction was not serious.

In contrast to the fall applications of M-3766, the spring application was an environmental disaster. The application date was May 19, 1973. The spring was wet and the soil was saturated with water at the time of application. Lateral movement of 3-5ft of picloram was determined using both the potato and soybean bioassay and up to 15-20 ft using the more sensitive Basswood bioassay. Trees and shrubs adjacent to the sprayed area showed extensive injury (Fig. 19) and many Tilia americana (Basswood) and Acer saccharinum (Hard Maple) trees were killed outright by root uptake of the materials even though not directly oversprayed at the 2 lb/A rate. Grass and other vegetation

Figure 18. Injury to soybeans in a field adjacent to a test area treated in early October, 1972 with 4 lb/A of M-3766. The area received water draining from about 1 mile of test plot and in the low spot in the center of the field, the injury occurred. This was the only instance of serious injury to non-target vegetation from a fall application of M-3766 in two years of testing. Photographed July 18, 1973.

Figure 19. A Sassafras tree adjacent to an area treated with 2 lb/A of M-3766 on May 19, 1973. The tree was not killed but showed considerable injury and malformed growth the first year after application. Photographed July 18, 1973. The following year, the tree leafed out normally. This response pattern was characteristic of all tree species in the spring test. Those not killed outright, survived.

in the treated plots accumulated the herbicide producing grass clippings lethal to sensitive species. The spring application of this material was deemed environmentally unsafe from the standpoint of injury to non-target vegetation and was not tested further.

Summary

Picloram, a component of M-3766, is the most potent and long-lived of the herbicides tested in this study. Used unwisely, it can have disastrous effects on non-target vegetation. Spring applications on water-soaked soil caused serious injury to desirable species in near proximity to the treated area. Fall applications of the same material resulted in no serious environmental side effects to non-target vegetation at practical rates of application (½ - 2 lb/A). Injury was not noted on vegetation not oversprayed directly. Insignificant quantities of the herbicide entered water either from drift at the time of spraying, leaching, or runoff. Tests of soil samples showed the herbicide to remain on the target area and biodegrade at the expected rate. Within one year after application, soil residues dropped below the phytotoxicity level (no longer injurious to the growth of soybeans) at the ½ and 1 lb/A rate and after 2 years for the 2 lb/A rate. Fall applications of M-3766 have the important advantage of environmental safety. Problems of drift are eliminated since the growing season is over. By the following spring, soil residues are largely dissipated and desirable trees and shrubs are becoming dormant and escape the herbicide unless oversprayed directly.

Acknowledgements

The applications of M-3766 were with truck-mounted equipment using personnel and vehicles generously provided by Chemitrol, Indianapolis, Indiana. The cooperation of the Joint Highway Research Project, Purdue University in sponsoring the major costs of these studies is gratefully acknowledged. I thank Jane Eberle and Steve Gratzer for assistance.

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EVALUATION OF POTENTIAL INJURY TO NON-TARGET VEGETATION FROM DRIFT OF SPRAY DROPLETS DURING HERBICIDE APPLICATION

Introduction

Drift is the term used for the movement of spray particles from the time they leave the application equipment until the time they hit the ground. Drift can be avoided or eliminated in the following ways:

a) Avoid the generation of fine spray particles

b) Avoid spraying in windy weather

c) Keep equipment close to target

d) Use early or late season applications when susceptible crops are not growing

e) Special drift reduction techniques such as invert imulsions, thickeners or gels or foams.

Basically, elimination of drift depends on elimination of fine particles in the spray (lower pressures and increased spray volume by employing a larger nozzle tip), proper conditions of application (wind is the major offender) and wise use of drift control agents. Drift is both wasteful of chemical and a hazard to the environment.

In this study, the effect of chemical drift was monitored in a soybean field in Tippecanoe County, Indiana at the request of the chemical applicator. Although nothing was learned that was not already known, the study serves as an example of the type of economic and environmental damage which can occur from the careless or improper application of brush-killing chemicals.

Materials and Methods

The field under study was located adjacent to a county road and the brush along the roadside was sprayed in mid-July with a mixture containing standard proportions of 2,4-D and 2,4,5-T. By the applicators own admission, the spraying operation was carried out with a strong wind which carried the spray particles by drift into the soybean field. The affected portion was approximately 0.8 mile. Evaluations were in late October approximately 3 months after spraying.

Results and Discussion

The appearance of the soybean plants (Fig. 20) confirms herbicide injury. Adventitious roots were prevalant over long regions of the stem. Herbicide injury extended for approximately 16 rows (Figs. 21 and 22) as deduced from the presence of these roots on the stem and the reduced height of the plants. Beans from approximately 3 ft of row were harvested from each of the affected rows and compared to unsprayed soybeans from other parts of the field.

Yield was cut heavily in the first 6 rows adjacent to the treated area to about 50% of expected. Yield from the next ten rows averaged a 35% reduction. The yield from portions of the field not damaged by herbicide was 47 bu/A; the total yield reduction due to negligence in applying the herbicide was 91 bushels of soybeans or about 114 bushels per mile.

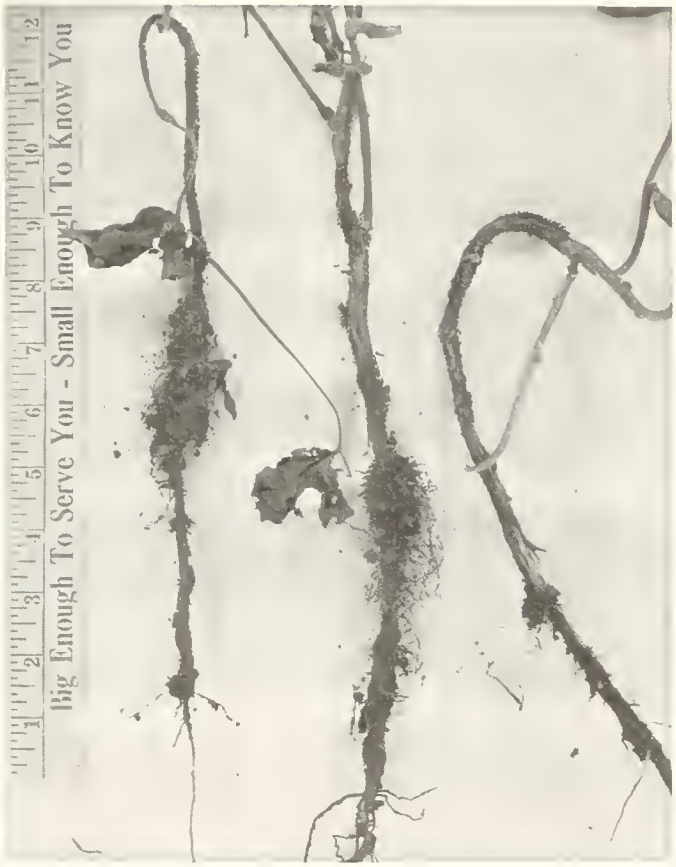

Figure 20. Soybean plants injured by spray drift from a brush spraying operation in Tippecanoe County, Indiana. A then standard application of an equal mixture of 2,4-D and 2,4,5-T was applied in mid July. Damage was evaluated in late October. The numerous adventitious roots on the stems are clear symptoms of injury from the herbicides used.

Figure 21. As in Figure 20, except comparing plants from rows 1, 3, 7 and 16 beginning adjacent to the sprayed area.

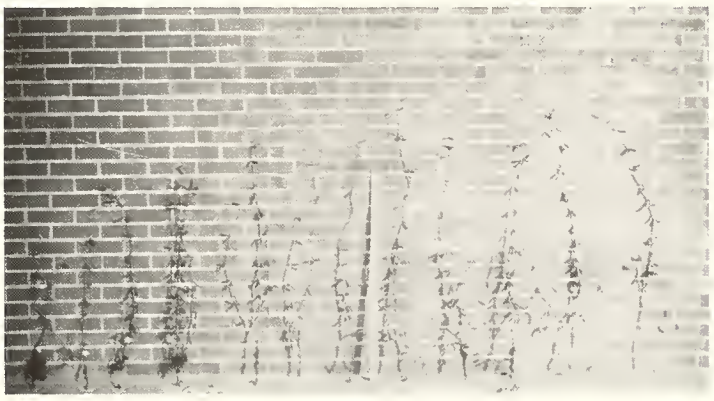

Figure 22. As in Figure 21, from left to right, plants from rows 1, 2, 3, 4, 6, 8, 10, 12, 14, 16 and two normal plants.

Summary

As shown by the preceding example obtained under actual use (or misuse) conditions, environmental damage from spray drift from herbicide applications to control brush can be costly. Reductions in yield to soybeans and other agricultural crops can be evaluated in economic terms; other forms of damage cannot. Drift can be avoided by appropriate application techniques.

Careful monitoring of spraying operations involving mid-summer applications, especially along county roads, should be encouraged to prevent drift and possible injury to non-target vegetation.

TOXICITY STUDIES OF NALCOTROL--A WATER-SOLUBLE DRIFT CONTROL AGENT

Because of importance in keeping herbicides on the target area and the prevention of drift, a number of drift control agents and drift control systems were evaluated in this project. By my judgement, a system that was effective and with no obvious environmental hazards was the use of Nalco-Trol (Nalcotrol), a product manufactured by the Nalco Chemical Company.

According to information supplied by the manufacturer, Nalcotrol has a polyacrylamide base of 22 million molecular weight. It is stable, does not degrade with shear and is biodegradable. It can be used with both aerial application or standard ground equipment. No special nozzles are required, visibility is improved while spraying and the cost of the material is less than $0.01 per gallon of spray solution. The oral toxicity to albino rats is negligible, the LD_{50} is in excess of 34,600 mg/kg body weight. Fish toxicity LD_{50}, 96 hours, is about 1,000 ppm with trout and blue gill being the species evaluated. Toxicity data on algae

This portion of the project was devoted to a superficial examination of the toxicity of Nalcotrol to green sunfish and mixed algal populations (Table 21). Procedures were as described for evaluation of herbicide toxicity. Nalcotrol was activated in water and fed as an aqueous solution with dosage being expressed as a function of Nalcotrol active.

Table 21. Algal genera tested

Division Cyanophyta

Order Oscillatoriales

Oscillatoria

Order Chroococcales

Microcystis

Division Chlrophyta

Order Volvocales

Eudorina

Order Ulotricales

Microspora

Stigeoclonium

Order Cladophorales

Pithophora

Order Zygnematales

Spirogyra

Cosmarium

Desmidium

Division Euglenophyta

Order Euglenales

Euglena

Trachelomonas

Division Chrysophyta

Order Centrales

Melosira

Order Pennales

Navicula

Plus the aquatic plants *Lemna minor* (Duckweed) and *Potamogeton crispus* (Curlyleaf Pondweed).

Green sunfish (Lepomis cyanellus) were tested at concentrations of 4, 0.4 and 0.04 ounces of Nalcotrol/100 gal. The fish in the 4 oz/100 gal dosage were immobilized within 30 minutes and were dead within 24 hours. Fish at 0.4 ounces of Nalcotrol/100 gal (= approximately 1000 ppm of Nalcotrol active assuming the formulated product is 1/3 active ingredient), survived and appeared normal during a one week observation period. Apparently, at the highest concentration, the gills became coated and the fish may have suffocated.

When Nalcotrol was added to optimal media at the dosage rate of 4 oz/100 gal, none of the algal species or general listed in Table 21 were affected. Similarly, the organisms were not affected by the lower dosages of 0.4 and 0.04 oz./100 gal. Nalcotrol is not phytotoxic.

Nalcotrol, used at the rate of 6-8 oz/100 gal of spray solution is an effective, easy to use, and safe agent to control or reduce misting and drift in spray applications for mid-summer treatments. With the use of a drift control agent, more of the spray solution reaches the vegetation to be controlled and is retained on the target area. Less drifts off onto adjacent cropland where the material is wasted or may be injurious to desirable vegetation.

FIELD EVALUATIONS OF ENVIRONMENTALLY-SAFE BRUSH CONTROL AGENTS

Introduction

Commercially available herbicides, either demonstrated or claimed to be without hazard to man or his environment, were evaluated for efficacy in control of Indiana brush species under actual or simulated conditions of field application. These studies were initiated in the fall of 1973, were continued in 1974 and 1975, and additional studies are planned for 1976.

Materials and Methods

Test sites were located in Boone, Kosciusko, Hancock, Tippecanoe, and White counties. Applications were with truck-mounted equipment operated by cooperating contractors or industrial applicators.

Standard industrial evaluations were used to determine the brush control efficacy and included % defoliation, % root collar resprouts, % with green stems, % dead for each species present, as well as effects on grass and other non-target vegetation. All treatments were evaluated in the second, and, if possible, third years to establish long-term efficacy. Comparisons were relative to a standard foliar application of 2,4,5-T + 2,4-D (see Introduction).

Results and Discussion

Tests where evaluations are complete or in progress are summarized on the pages which follow. Control, by species, are listed for each of the test plots. For these tests, as well as those still in progress, summaries are provided at the end of the report.

BRUSH CONTROL TEST # 9-73-K Date: September 17, 19 73

Cooperator: The Daltons, Warsaw, Indiana Applied by: The Daltons, Warsaw, Indiana

Location: Kosciusko County 1000E from 800N to 1300N

Plot Size: 0.25-2 miles X 10 ft. No. of Replications: 1-5

Soil pH: N/A Organic %: N/A Type Treatment: Simulated Aerial

Spray Vol.: 40-70 gpa Spray Pressure: N/A psi Nozzle Type: Turrent

Type Spray Equipment: Truck-Mounted

Temperature at Application	[X]	hot	[ ]	moderate	[ ]	cool
Soil Moisture:	[X]	wet	[ ]	optimum	[ ]	dry
Wind Velocity:	[ ]	strong	[X]	slight	[ ]	none
Soil Type:	[ ]	heavy	[ ]	medium	[ ]	light
Humidity:	[ ]	high	[ ]	medium	[ ]	low

Recent or Current Herbicide Usage on Test Area: None

Treatment	Formulation, Batch number	Dosage: Lbs.	Plot Numbers – Reps. 1	2	3	4
Untreated	...	...	1B	2B		
Banvel 4 WS	(4.4 lb/A)	6.7 lb/100 gal	1A	2A		
Banvel 520 (3 lb/A (2 gal/100 gal) + Accutrol (1 gal/100 gal)			3	4	5	6
	+ Oil	(37.5 gal/100 gal)	7			
Banvel 520 + Accutrol + Oil (as above)		6 lb/A	8 (weed control only)			
2,4-D + 2,4,5-T (3 qts/100 gal)		1½ lb + 1½ lb	9*			
Banvel 520 + Accutrol + Oil (½ gal + 1 gal + 15 gal) + 2,4,5-T			10**			
(1 gal/100 gal), 82 gal water						

* Applied in early June. **Applied in mid-November.

Species	Stage of Growth	Height	Species	Stage of Growth	Height
Cottonwood	***	8-12'	Black Walnut		3-15'
Crabapple		8'	Choke Cherry		3-5'
Blackhaw		8'	Ash		3-25'
American Elm		4-20'	Sycamore		15'
Sumac		5'	Hackberry		8-12'
Sassafras		5'	Russian Olive		5'
Red Haw (Crataegus)		6-20'	Black Cherry		5-25'
Red-Stemmed Dogwood		4-5'	Red Mulberry		8-12'
Sugar Maple		3-25'	Hickory		3-8'
Silver Maple		3-25'	Basswood		3-25'

***All species were in full leaf, abscission layers not yet formed

BRUSH CONTROL TEST # 9-73-K

Species Continued

Chestnut Oak	4-20'
Bur Oak	4-15'
Box Elder	15-20'
Red Cedar	4'
Buckeye	5-6'
Black Locust	4-6'
Wild Grape	
Greenbriar	
Poison Ivy	
Virginia Creeper	
Multiflora Rose	
Black Raspberry	
Blackberry	
Bittersweet	
Elderberry	

EVALUATION REPORT

BRUSH CONTROL TEST # 9-73-K

Application Date: September 17, 1973

Evaluation Date: May 21, 1974

Location: Kosciusko County

~~1000 E from 800 N to 1300 N~~

Evaluator: D. J. Morré; Lex Dalton

Plot No.	Species	Treatment Rating*
1A	Cottonwood	0
	Crabapple	1
	Blackhaw	1
	American Elm	2-4
	Sumac	3-5
	Sassafras	5
	Red Haw	3
	Wild Grape	0
	Greenbriar	1
	Dogwood	1.5
	Sugar Maple	0
	Walnut	1
	Chokecherry	0
	Ash	1-4
	Multiflora Rose	0
	Virginia Creeper	0
	Sycamore	1
	Hackberry	1
	Willow	4-5
	Russian Olive	5
	Raspberry	0

Evaluation of Surviving Species
May 26, 1975

Plot No.	Species	Treatment Rating*
1A,B	Ash	2-5
	Elm	0-3
	Mulberry	0-2
	Sassafras	0-3
	Sumac	3-5
	Greenbiar	0
	Willow	0-5
	Raspberry	1
	Hard Maple	0
	Russian Olive	5
	Bittersweet	0
	Dogwood	0
	Wild Cherry	0
	Walnut	0
	Wild Rose	0
	Wild Grape	0
	Multiflora Rose	0
	Cottonwood	0

*0 = No effect
1 = Root Collar resprouts
2 = Less than 50% defoliation
3 = More than 50% defoliation
4 = 100% defoliation with green stems
5 = Dead

EVALUATION REPORT

BRUSH CONTROL TEST # 9-73-K

Application Date: September 17, 1973

Evaluation Date: May 21, 1973

Location. Kosciusko County

1000 E from 800 N to 1300 N

Evaluator: D. J. Morré; Lex Dalton

Plot No.	Species	Treatment Rating*
2A	Black Cherry	3
	Ash	3
	Sugar Maple	0
	Cottonwood	0
	Willow	1
	Raspberry	0
3	Sugar Maple	0
	Black Cherry	0-4
	Mulberry	0
	Multiflora Rose	0-1
	American Elm	2-3
	Willow	2
	Ash	4
	Hickory	0
	Black Walnut	1
	Basswood	1
	Chestnut Oak	1
	Bur Oak	4

Evaluation of Surviving Species
May 26, 1975

Plot No.	Species	Treatment Rating*
3,4	Walnut	0
	Wild Rose	0
	Hazel	1
	Wild Grape	0-5
	Multiflora Rose	1
	Cottonwood	1
	Trumpetvine	0
	Basswood	1
	Choke Cherry	2
	Buckeye	0
	Apple	0
	Osage Orange	0

*0 = No effect
1 = Root Collar resprouts
2 = Less than 50% defoliation
3 = More than 50% defoliation
4 = 100% defoliation with green stems
5 = Dead

EVALUATION REPORT

BRUSH CONTROL TEST # 9-73-K

Application Date: 17 September 1973 Evaluation Date: May 21, 1974

Location. Kosciusko County Evaluator: D. J. Morré; Lex Dalton

1000 E from 800 N to 1300 N

Plot No.	Species	Treatment Rating*
4	American Elm	2
	Ash	4
	Virginia Creeper	0
	Hard Maple	0
	Multiflora Rose	0
	Black Cherry	0
5	Elderberry	0
	Black Cherry	1
	Dogwood	2
	Elm (American)	2
	Blackberry	0
	Cottonwood	0
	Sassafras	1
	Crabapple	2
	Sumac	3
	Mulberry	3
	Hackberry	3
	Elderberry	5
	Box Elder	4

Evaluation of Surviving Species
May 26, 1975

Plot No.	Species	Treatment Rating*
3-4	Ash	2.3
	Elm	2
	Mulberry	1.3
	Hackberry	1
	Sassafras	3
	Sumac	3.3
	Poison Ivy	5
	Greenbriar	0
	Black Locust	4
	Willow	2
	Raspberry	1
	Box Elder	1
	Hard Maple	0
	Soft Maple	1
	Hickory	0
	Chestnut Oak	3
	Swamp White Oak	0
	Red Oak	0
	Honey Locust	0
	Elderberry	2
	Dogwood	2.5
	Wild Cherry	1
	Crab Apple	5
	Wild Plum	2
	Red Haw	3

*0 = No effect
1 = Root Collar resprouts
2 = Less than 50% defoliation
3 = More than 50% defoliation
4 = 100% defoliation with green stems
5 = Dead

EVALUATION REPORT

BRUSH CONTROL TEST # 9-73-K

Application Date: 17 September 1973

Evaluation Date: May 21, 1974

Location: Kosciusko County

~~1000 E from 800 N to 1300 N~~

Evaluator: D. J. Morré; Lex Dalton

Plot No.	Species	Treatment Rating*	Evaluation of Surviving Species
6	Red Cedar	3	
	Ash	5	
	Mulberry	3	
	Buckeye	0	
	Silver Maple	0	
	Hard Maple	0	
	Ash	2-3	
	Bittersweet	2	
	Dogwood	5	
	Elm	2	
	Red Haw	2	
	Black Locust	2	
7	Poison Ivy	3	
	American Elm	2-3	
	Virginia Creeper	0	
	Choke Cherry	0	
	Sassafras	1-5	
	Black Cherry	0-1	
	Locust	2	
	Mulberry	0	

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1 = Root Collar resprouts
2 = Less than 50% defoliation
3 = More than 50% defoliation
4 = 100% defoliation with green stems
5 = Dead

EVALUATION REPORT

BRUSH CONTROL TEST # 9-73-K

Application Date: 17 September 1973

Evaluation Date: May 21, 1974

Location: Kosciusko County

1000 E from 800 N to 1300 N

Evaluator: D. J. Morré; Lex Dalton

Plot No.	Species	Treatment Rating*	Evaluation of Surviving Species
7 Cont.	Linden	1	
	Hickory	0	
	Multiflora Rose	0-1	
	Black Walnut	1	
9	Poison Ivy	3-4	
	American Elm	1-2	
	Viroinia Creeper	0	
	Chcke Cherry	2	
	Sassafras	0	
	Black Cherry	0	
	Locust	3	
	Mulberry	0	
	Basswood	2	
	Sumac	2	
	Hickory	0	
	Multiflora Rose	0	
	Walnut	0	

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2 = Less than 50% defoliation
3 = More than 50% defoliation
4 = 100% defoliation with green stems
5 = Dead

EVALUATION REPORT

BRUSH CONTROL TEST # 9-73-K

Application Date: 17 September 1973

Evaluation Date: May 21, 1974

Location: Kosciusko County

1000 E from 800 N to 1300 N

Evaluator: D. J. Morré; Lex Dalton

Plot No.	Species	Treatment Rating*	Evaluation of Surviving Species
10	Black Cherry	5	
	Ash	4-5	
	Red Haw	4	
	Chestnut Oak	5	
	Elm	3	
	Raspberry	5	

*0 = No effect
1 = Root Collar resprouts
2 = Less than 50% defoliation
3 = More than 50% defoliation
4 = 100% defoliation with green stems
5 = Dead

BRUSH CONTROL TEST # 9-73-TMc Date: 13 September , 19 73

Cooperator: Chemitrol, Indianapolis, Ind. Applied by: Chemitrol, Indianapolis, Ind

Location: Tippecanoe County, Indiana, McCormick Gravel Road

Plot Size: 0.25 mile X 15 ft. No. of Replications: 3

Soil pH: N/A Organic %: N/A Type Treatment: Handoun

Spray Vol.: 50 gpa Spray Pressure: 65 psi Nozzle Type: OC

Type Spray Equipment: Truck-Mounted

Temperature at Application	☐	hot	☒	moderate	☐	cool
Soil Moisture:	☒	wet	☐	optimum	☐	dry
Wind Velocity:	☐	strong	☒	slight	☐	none
Soil Type:	☐	heavy	☐	medium	☐	light
Humidity:	☐	high	☐	medium	☐	low

Recent or Current Herbicide Usage on Test Area: None

Treatment	Formulation, Batch number	Dosage: Lbs.	Plot Numbers – Reps. 1	2	3	4
Untreated	...	...	7	8	9	
Banvel + 2,4-D*		1 lb + 2 lb/A	1	3	5	
Banvel*	1gal/100 gal	2 lb/A	2	4	6	

* Plus Nalcotrol, a drift control agent, 8 oz/100 gal.

Species	Stage of Growth	Height	Species	Stage of Growth	Height
American Elm	**	4-20'	Ash		3-25'
Honey Locust		12'	Willow		6'
Mulberry		8'	Sycamore		25'
Hickory		6-30'	Cottonwood		8-12'
Hazel Nut		8'	Sugar Maple		3-25'
Hackberry		10-15'	Catalpa		15'
Wild Cherry		10-25'	Sassafras		7'
Apple		20'	Bur Oak		8-25'
Crataegus (Red Haw)		8-15'	Water Oak		8-25'
Dogwood (Red-Stemmed)		4-5'	Chinquapin Oak		8-25'
Black Walnut		10-25'			

** All species were in full leaf, abscission layers not yet formed.

BRUSH CONTROL TEST # 9-73-TMc

Species Continued

Sumac	5-7'
Pine	15'
Redbud	3-25'
Multiflora Rose	
Blackberry	
Elderberry	
Wild Grape	
Poison Ivy	
Trumpet Vine	
Virginia Creeper	

EVALUATION REPORT

BRUSH CONTROL TEST # 9-73-TMc

Application Date: 13 September, 1973 Evaluation Date: 11 June 1974

Location: Tippecanoe County, Indiana
McCormick Gravel Road

Evaluator: D. J. Morré

Plot No.	Species	Treatment Rating*	Evaluation of Surviving Species
1	Poison Ivy	0	
	Virginia Creeper	0	
	American Elm	1-0	
	Honey Locust	0	
	Mulberry	0	
2	Black Cherry	0	
	Dogwood	0	
	American Elm	0	
	Willow	0	
	Poison Ivy	0	
	Sycamore	0	
	Cottonwood	0	
	Sugar Maple	0	
	Elderberry	0	
3	American Elm	0	
	Trumpet Vine	1	
	Hickory	0	
	Hazelnut	0	
	Blackberry	1	
	Wild Cherry	0-1	

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2 = Less than 50% defoliation
3 = More than 50% defoliation
4 = 100% defoliation with green stems
5 = Dead

EVALUATION REPORT

BRUSH CONTROL TEST # 9-73-TMc

Application Date: 13 September, 1973

Evaluation Date: 11 June 1974

Location: Tippecanoe County, Indiana
McCormick Gravel Road

Evaluator: O. J. Morré

Plot No.	Species	Treatment Rating*	Evaluation of Surviving Species
3 Cont.	Apple	1	
	Crateous	2	
	Poison Ivy	0-5	
	Dogwood	0	
	Grape	0	
	Walnut	3	
4	Black Locust	0	
	Black Cherry	0-2	
	Wild Grape	0	
	Catalpa	0	
	Poison Ivy	0-2	
	American Elm	3-5	
	Sassafras	0	
	Bur Oak	0	
	Hazelnut	0	
	Hickory	0-5	
	Multiflora Rose	0	
	Dogwood	0	
	Water Oak	0-1	
	Blackberry	0	
	Chinquapin Oak	4	
	Elderberry	5	

*0 = No effect
1 = Root Collar resprouts
2 = Less than 50% defoliation
3 = More than 50% defoliation
4 = 100% defoliation with green stems
5 = Dead

EVALUATION REPORT

BRUSH CONTROL TEST # 9-73-IMc

Application Date: 13 September, 1973

Evaluation Date: 11 June 1973

Location: Tippecanoe County, Indiana
McCormick Gravel Road

Evaluator: D. J. Morré

Plot No.	Species	Treatment Rating*	Evaluation of Surviving Species
5	American Elm	0-1	
	Blackberry	0-1	
	Poison Ivy	0-2	
	Black Cherry	0	
	Multiflora Rose	0	
	Wild Grape	0	
	Ash	2	
	Elderberry	5	
6	Mulberry	0	
	American Elm	0-2	
	Hickory	4	
	Osage Orange	0-2	
	Blackberry	2	
	Multiflora Rose	0-2	
	Sumac	0-2	
	Pine	3	
	Redbud	0	
	Red Haw	0-1	
	Hackberry	4	
	Water Oak	0-2	

*0 = No effect
1 = Root Collar resprouts
2 = Less than 50% defoliation
3 = More than 50% defoliation
4 = 100% defoliation with green stems
5 = Dead

BRUSH CONTROL TEST # 9-73TCfGh Date: September 14, 19 73

Cooperator: Chemitrol, Indianapolis, Ind. Applied by: Chemitrol, Indianapolis, Ind.

Location: Tippecanoe County, Indiana, County Farm Road and Greenhill

Plot Size: 0.25 mile X 15 feet No. of Replications: 2

Soil pH: N/A Organic %: N/A Type Treatment: Handgun

Spray Vol.: 50 gpa Spray Pressure: 65 psi Nozzle Type: OC

Type Spray Equipment: Truck-Mounted

Temperature at Application	☐	hot	☒	moderate	☐	cool
Soil Moisture:	☒	wet	☐	optimum	☐	dry
Wind Velocity:	☐	strong	☒	slight	☐	none
Soil Type:	☐	heavy	☐	medium	☐	light
Humidity:	☐	high	☐	medium	☐	low

Recent or Current Herbicide Usage on Test Area: None

Treatment	Formulation, Batch number	Dosage: Lbs.	Plot Numbers – Reps. 1	2	3	4
Untreated	---	---	1	4		
Banvel + 2,4-D*		1 lb + 2 lb/A	2	5		
Banvel*		2 lb/A	3	6		

* Plus Nalcotrol, an agent to control drift, 8 oz./100 gal.

Species	Stage of Growth	Height	Species	Stage of Growth	Height
Red-stemmed Dogwood	**	4-5'	Silver Maple		3-25'
Black Cherry		9-25'	Ash		2-25'
Water Oak		8-25'	Hackberry		8-15'
Chinquapin Oak		8-25'	Wild Grape		
Honey Locust		12-30'	Poison Ivy		
Mulberry		15'	Greenbriar		
Black Cherry		12-25'			

**All species were in full leaf, abscission layers not yet formed.

EVALUATION REPORT

BRUSH CONTROL TEST # 9-73-ICfGh

Application Date: September 14, 1973

Evaluation Date: 13 June 1974

Location: Tippecanoe County, Indiana
County Farm Road and Greenhill

Evaluator: D. J. Morré

Plot No.	Species	Treatment Rating*	Evaluation of Surviving Species
2	All species	0	
3	Red-stemmed Dogwood	5	
	Black Cherry	2-5	
	Water Oak	4-5	
	Poison Ivy	2	
	Greenbriar	1	
	Dogwood	3	
	Chinquapin Oak	3	
5	All species	0	
6	Honey Locust	4-5	
	Mulberry	1	
	Black Cherry	4	
	Silver Maple	0	
	Ash	4	
	Hackberry	1	
	Wild Grape	0	
	Poison Ivy	2	
	Red-stemmed Dogwood	5	
	Honey Locust	4	
	Water Oak	4	

*0 = No effect
1 = Root Collar resprouts
2 = Less than 50% defoliation
3 = More than 50% defoliation
4 = 100% defoliation with green stems
5 = Dead

BRUSH CONTROL TEST # 6-74-B Date: June 1, 19 74

Cooperator: Chemitrol, Indianapolis, Ind. Applied by: Chemitrol, Indianapolis, Ind.

Location: Boone County Indiana. Rep. I: SR 32, ½ mile W of I-65. Rep. II. Sam Ralston Rd. off SR 32 east of jct. I-65. Rep. III: Hazelrig Ave off U.S. 52

Plot Size: 0.25 Mile X 20 ft. No. of Replications: 3

Soil pH: N/A Organic %: N/A Type Treatment: Sprino Foliage

Spray Vol.: 90 gpa Spray Pressure: 65 psi Nozzle Type: OC (Handgun)

Type Spray Equipment: Truck Mounted

Temperature at Application	☐ hot	☒ moderate	☐ cool
Soil Moisture:	☐ wet	☒ optimum	☐ dry
Wind Velocity:	☐ strong	☒ slight	☐ none
Soil Type:	☐ heavy	☐ medium	☐ light
Humidity:	☐ high	☐ medium	☐ low

Recent or Current Herbicide Usage on Test Area: Sprayed about 5 years previously with a mixture of 2,4-D and 2,4,5-T by helicopter

Treatment	Formulation, Batch number	Dosage: Lbs.	Plot Numbers – Reps. 1	2	3	4
Untreated	...	...	1	3	5	
Banvel (3 gal/300 gal water)*		3.6 lb/A	2	4	6	

Species	Stage of Growth	Height	Species	Stage of Growth	Height
Multiflora Rose	**	3-5ft	Apple	**	8-12
Willow		3-5ft	Box Elder		6-25
Mulberry		3-15	Hard Maple		6-15
Wild Plum		2-10	Black Cherry		3-15
Honey Locust		5-15	Elm		1-15
Red Haw		5-15	Trumpet Vine		4
Elderberry		5	Ash		4-15

**All species were in full leaf.

EVALUATION REPORT

BRUSH CONTROL TEST # 6-74-B

Application Date: June 1, 1974 Evaluation Date: May 20, 1975

Location: Boone County, Indiana Evaluator:

SR 32, ½ mile W of I-65; Sam Ralston Rd off SR 32 E of jct. with I-65 and Hazelrig Ave off U.S. 52

Plot No.	Species	Treatment Rating*
2	Multiflora Rose	5
	Willow	3
	Mulberry	1
	Wild Plum	2
	Honey Locust	5
	Red Haw	2
	Elderberry	5
	Apple	1
	Box Elder	5
	Hard Maple	5
4	Black Cherry	3-5
	Black Locust	5
	Wild Plum	5
	Box Elder	5
	Mulberry	0
	Elderberry	5
	Hard Maple	5
	Elm	1-2
6	Box Elder	5
	Trumpet Vine	3
6 Cont.	Red Haw	1
	Wild Plum	3-5
	Elm	3-5
	Green Briar	0
	Raspberry	1
	Willow	3
	Wild Cherry	4
	Ash	5

*0 = No effect
1 = Root Collar resprouts
2 = Less than 50% defoliation
3 = More than 50% defoliation
4 = 100% defoliation with green stems
5 = Dead

BRUSH CONTROL TEST # 9-74-G Date: September 4, 1974

Cooperator: State of Indiana Applied by: Clyde Mason

Location: Hancock County, Indiana, East of Greenfield, Along Old U.S. 40

Plot Size: 1-2 miles X 25 ft. No. of Replications: 3

Soil pH: N/A Organic %: N/A Type Treatment: Foliage (Fall)

Spray Vol.: 30-60 gpa Spray Pressure: 65 psi Nozzle Type: 300 OC

Type Spray Equipment: Truck Mounted Single Jet plus Boom

Temperature at Application	☐ hot	☒ moderate 63 F	☐ cool
Soil Moisture:	☐ wet	☒ optimum	☐ dry
Wind Velocity:	☐ strong	☐ slight	☒ none
Soil Type:	☐ heavy	☐ medium	☐ light
Humidity:	☐ high	☒ medium	☐ low

Recent or Current Herbicide Usage on Test Area: 2,4-D 3.3 lb/A fall 1972 and spring 1973

Treatment	Formulation, Batch number	Dosage: Lbs.	Plot Numbers – Reps. 1	2	3	4
Untreated	...	...	19	20	21	
Silvex (2,4,5-TP)	60 gpa	6 lb/A	1	2	3	
		3 lb/A	4	5	6	
M-3766 (Picloram + 2,4-D 3:1)	30 gpa	1.5 lb/A	7	8	9	
		2 lb/A	10	11	12	
Banvel (dicamba)	50 gpa	5.5 lb/A	13	14	15	
		2.25 lb/A	16	17	18	

Species	Stage of Growth	Height	Species	Stage of Growth	Height
Mulberry	**	3-12	Ash	**	4-20
Black Locust		2-15	Box Elder		4-20
Hackberry		5-15	Catalpa		12
Poison Ivy		3-5	Elderberry		5
Elm		3-10	Multiflora Rose		3-5
Silver Maple		4-20			
Black Cherry		3-20			

** All species were in full leaf, abscission layers not yet formed.

EVALUATION REPORT

BRUSH CONTROL TEST # 9-74-G

Application Date: September 4, 1974 **Evaluation Date:** May 20, 1975

Location: Hancock County, Indiana, East of Greenfield, along Old U.S. 40 **Evaluator:** Morre and Eberle

Plot No.	Species	Treatment Rating*
Banvel 2¼ lb/A	Mulberry	0
	Poison Ivy	0
	Elm	0
	Black Cherry	0
	Ash	2
Banvel 5½ lb/A	Mulberry	2
	Poison Ivy	2
	Red-stemmed Dogwood	3
Silvex 3 lb/A	Mulberry	2
	Black Locust	5
	Hackberry	3
	Ash	0
Silvex 6 lb/A	Mulberry	2
	Black Locust	5
	Hackberry	1
	Poison Ivy	3
	Elm	5
	Ash	2-3
	Wild Grape	3
	Red-stemmed Dogwood	3
M-3766 1½ lb/A	Black Locust	5
	Hackberry	5
	Elm	4
	Ash	1
	Elderberry	5
	Multiflora Rose	5
M-3766 2 lb/A	Elm	3
	Silver Maple	5
	Ash	2
	Box Elder	3
	Catalpa	5
	Elderberry	5

*0 = No effect
1 = Root Collar resprouts
2 = Less than 50% defoliation
3 = More than 50% defoliation
4 = 100% defoliation with green stems
5 = Dead

BRUSH CONTROL TEST # 9-74-W Date: September 10 , 19 74

Cooperator: The Daltons, Inc. Warsaw Applied by: The Daltons, Inc. Warsaw

Location: Kosciusko County Wooster Rd; Old SR 30 and SR 13

Plot Size: 1-3 miles X 8-20 ft. No. of Replications: _____

Soil pH: _____ Organic % : _____ Type Treatment: Foliage (Fall)

Spray Vol.: 40 gpa Spray Pressure: _____ psi Nozzle Type: Broadjet 150 OC and Hand Gun

Type Spray Equipment: Truck Mounted

Temperature at Application	☐ hot	☒ moderate	☐ cool
Soil Moisture :	☐ wet	☒ optimum	☐ dry
Wind Velocity:	☐ strong	☐ slight	☒ none
Soil Type:	☐ heavy	☐ medium	☐ light
Humidity:	☐ high	☐ medium	☐ low

Recent or Current Herbicide Usage on Test Area: Not recently sprayed

Treatment	Formulation, Batch number	Dosage: Lbs.	Plot Numbers – Reps. 1	2	3	4
Untreated	...	...	1	4	7	
3-Way Mixture 2½ gal 2,4-D	+ 2½ gal Banvel	3 lb/A	2			
+ 2½ gal 2,4,5-TP (Silvex)	in 400 gal water	6 lb/A	3			
M-3766 (1½ lb Tordon + ½ lb 2,4-D amine/gal)		1 lb/A	5			
5 gal in 400 gal water		2 lb/A	6			

Species	Height	Species	Height	Species	Height	Species	Height
Ash	3-20ft	Poison Ivy	1-3 ft	Soft Maple	3-20	Honey Locust	5-15
Elm	3-25	Greenbiar	5	Hickor	5	Catalpa	12-15
Blackberry	3-5	Black Locust	3-15	Black Oak	5-25	Elderberry	3-5
Mulberry	5-12	Willow	3-15	Willow Oak	5-25	Sassafras	3-15
Hackberry	8	Raspberry	3-5	Chestnut Oak	5-25	Bittersweet	5
Sassafras	1-15	Box Elder	3-20	Swamp White Oak	15	Dogwood	4-8
Sumac	2-5	Hard Maple	5-15	Red Oak	5-25	Cherry	2-18

All species were in full leaf; abscission layers were not yet formed.

List of Species Continued for Brush Control Test # 9-74-W

Crab Apple	5-12 ft
Wild Plum	5-8
Red Haw	5-8
Walnut	8-15
Wild Rose	1-5
Hazel	5
Wild Grape	variable
Multiflora Rose	3-6
Cottonwood	12
Trumpetvine	variable
Basswood	6-12
Choke Cherry	5-7
Buckeye	12
Apple	8
Osage Orange	8-15

EVALUATION REPORT

BRUSH CONTROL TEST # 9-74-W

Application Date: September 10, 1974 **Evaluation Date:** May 26, 1975

Location: Kosciusko County Wooster Rd and Old SR 30 and SR 13 **Evaluator:** Morre and Thornbury

Plot No.	Species	Treatment Rating*
2	Elm	5
	Blackberry	5
	Mulberry	4
	Hackberry	0
	Sumac	5
	Poison Ivy	0
	Greenbriar	0
	Black Locust	5
	Raspberry	1
	Box Elder	5
	Hard Maple	0
	Honey Locust	5
	Catalpa	5
	Elderberry	5
	Bittersweet	3
	Dogwood	5
	Wild Cherry	5
	Crab Apple	4-5
	Red Haw	2
	Walnut	0
	Wild Rose	5
	Hazel	5
	Wild Grape	2
	Multiflora Rose	5
	Cottonwood	3
3	Elm	3
	Blackberry	5
	Mulberry	3-5
	Sumac	5
	Poison Ivy	2
	Greenbriar	0
	Black Locust	5
	Raspberry	2
	Willow Oak	5
	Red Oak	0
	Honey Locust	3
	Elderberry	5
	Bittersweet	3
	Dogwood	5
	Wild Cherry	5

*0 = No effect
1 = Root Collar resprouts
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3 = More than 50% defoliation
4 = 100% defoliation with green stems
5 = Dead

EVALUATION REPORT

BRUSH CONTROL TEST # 9-74-W

Application Date: September 10, 1974

Evaluation Date: May 26, 1975

Location: Kosciusko County Wooster Rd and Old SR 30 and SR 13

Evaluator: Morre and Thornbury

Plot No.	Species	Treatment Rating*
3	Crab Apple	4
	Wild Plum	5
	Red Haw	5
	Walnut	0-5
	Hazel	5
	Cottonwood	3
5	Elm	5
	Blackberry	5
	Sassafras	2
	Sumac	5
	Black Locust	2
	Elderberry	5
	Dogwood	5
6	Elm	4
	Mulberry	3
	Greenbriar	5
	Black Locust	1
	Willow	5
	Elderberry	2
	Wild Plum	5
6	Red Haw	5
	Wild Rose	5

*0 = No effect
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3 = More than 50% defoliation
4 = 100% defoliation with green stems
5 = Dead

BRUSH CONTROL TEST # 9-75-K Date: September 16 , 19 75

Cooperator: Tippecanoe County Road Department Applied by: John Poulter

Location: Barton Beach Road at SR 25

Plot Size: about 0.25 mile No. of Replications: 3

Soil pH: _____ Organic %: _____ Type Treatment: Fall Foliage

Spray Vol.: _____ gpa Spray Pressure: _____ psi Nozzle Type: Broadjet 150 OC and Handgun

Type Spray Equipment: Truck Mounted

Temperature at Application	☐ hot	☐ moderate	☒ cool
Soil Moisture:	☒ wet	☐ optimum	☐ dry
Wind Velocity:	☐ strong	☐ slight	☒ none
Soil Type:	☐ heavy	☐ medium	☐ light
Humidity:	☐ high	☐ medium	☐ low

Recent or Current Herbicide Usage on Test Area: No recent use of herbicides known

Treatment	Formulation, Batch number	Dosage: Lbs.	Plot Numbers – Reps. 1	2	3	4
Untreated	---	---	1	3	5	
Krenite Brush Control Agent			2	4	6	
+ Surfactant						

Species	Stage of Growth	Height	Species	Stage of Growth	Height
Black Locust	**	8	Sassafras		3-8
Elm		3-8	Oak		5-20
Sumac		5	Silver Maple		6-20
Willow		3-8	Hawthorn		5-10
Ironwood		6	Wild Cherry		3-15
Beech		8-15	Honey Locust		5-15
Dogwood		3-6	Black Walnut		12

Species continued for 9-75-K

Hickory	6-15
Crab Apple	8
Wafer Ash	6
Green Ash	4-18
Hackberry	4-16
Mulberry	5-20
Poplar	8
Multiflora Rose	5
Box Elder	6-20
Basswood	8-20

Krenite gave good control of black locust, elm, sumac, willow, ironwood, beech, dogwood, sassafras and various oaks. Partial control and growth supression was obtained on silver maple, hawthorn, wild cherry, honey locust, black walnut, hickory, crab apple, wafer ash, green ash, hackberry, mulberry and poplar. Multiflora rose and box elder were ineffectively controlled. Basswood and linden were resistent.

Krenite seems to be an especially valuable herbicide for "trimming", an operation where the spray is directed to cover only the part of the tree to be controlled. This treatment is expected to better serve the needs for county roads where broad spectrum brush control is desired but damage to large, established trees and adjacent crops and other non-target species must be minimal.

Evaluations on June 13, 1976 showed: Elm 100% dead, Ash with small root sprouts, Box Elder 50% dead, Silver Maple, greater than 50% dead; Black Locust 100% dead, Honey Locust 50% dead, Crab Apple and Wafer Ash 50% dead, Willow 80 to 100% dead, Hackberry 90% dead, Linden or Basswood 0 to 25% defoliated, Ironwood and Beech 100% dead, Dogwood 100% dead, Green Ash 50-100% dead, Sassafras 100% dead, Hickory 25% dead and Multiflora Rose 0 to 50% dead.

BRUSH CONTROL TEST # 9-76-K Date: September, 19 76

Cooperator: The Daltons, Warsaw, Indiana Applied by: The Daltons, Warsaw, Indiana

Location: Kosciusko County, Indiana

Plot Size: About 1 mile = about 6 acres No. of Replications: 3

Soil pH:_____ Organic %: _____ Type Treatment: Microfoil Boom

Spray Vol.: _____ gpa Spray Pressure: _____ psi Nozzle Type: _____

Type Spray Equipment: Truck Mounted

Temperature at Application	☐ hot	☐ moderate	☐ cool
Soil Moisture:	☐ wet	☐ optimum	☐ dry
Wind Velocity:	☐ strong	☐ slight	☐ none
Soil Type:	☐ heavy	☐ medium	☐ light
Humidity:	☐ high	☐ medium	☐ low

Recent or Current Herbicide Usage on Test Area: No recent herbicide usage known.

Treatment	Formulation, Batch number	Dosage: Lbs.	Plot Numbers – Reps. 1	2	3	4
Untreated	...	...				
3-Way Mixture of dicamba +	2,4-D + Silvex	1.2 gal = 5 lb/A				
Krenite Brush Control Treatment + Surfactant		2 gal = 8 lb/A				
Will be compared to standard treatments of Brush Killer and						
Tordon applied to county roads in the same vicinity						

Species	Stage of Growth	Height	Species	Stage of Growth	Height
See Brush Control Test 9-73-K					

Some photographs of test plots are included in Figures 23-28. Generally sight distances were improved along county roads even under conditions where complete control of brush was not achieved, e.g. Krenite Brush Control Agent or higher rates of dicamba + 2,4-D.

Other Brush Control Tests and Evaluations

More limited tests were evaluated with the following materials.

Soil Sterilants- Hyvar X-L mixed at a rate of 1 gal/5 gal water and applied at the rate of 1 oz/2" basal diameter as a fall treatment at the crown. Good brush control was achieved; danger to adjacent crops would be expected to be minimal. The major problem with this and other soil sterilant treatments is that grass is killed (Fig. 29) leaving the soil open to erosion.

2,4-DP + 2,4,5-TP Mixtures- Evaluations were made of a ditch bordering U.S. 231 in White County. The application was by helicopter by a private contractor. Early control looked very good but regrowth was noted in subsequent years. This treatment has potential for use on county roads and plans are to begin more detailed evaluations in 1976.

Garlon- A new product of Dow Chemical Company currently available under an experimental label for non-crop uses will also be evaluated in 1976 as a possible replacement for the 3-Way Mixture on the Interstate System.

Krenite Brush Control Agent- Two additional tests with Krenite were evaluated in 1974 (Kosciusko County)(Fig. 28) (and on the Interchange between I-65 and I-465 on the north side of Indianapolis). Both were applied in the fall as recommended. In the Indianapolis test, partial control of sycamore was achieved with good control of willow, black cherry, and tree-of-heaven (Alianthus). Box elder was not controlled and partial kill was achieved for red mulberry and poison ivy.

Fig. 23. Application of experimental herbicides to brush using truck-mounted equipment belonging to a cooperating contractor. This particular equipment was supplied by:

Chemitrol, Indianapolis.

Other cooperators who were generous with time and equipment include:

The Daltons, Inc., Warsaw, Indiana

Clyde Mason, Greenfield Subdistrict, ISHD, Greenfield Indiana

Tippecanoe County Road Department

Kosciusko County Road Department

Charles Middleton, Velsicol Chemical Corporation

John Poulter, DuPont Chemical Company

Dr. J. B. Regan, Dow Chemical Company

Figure 24. Brush control test 9-73K in Kosciusko County, Indiana. Banvel 4 WS was applied at a rate of 4.4 lb/A in the fall (September 17, 1973) and was ineffective in achieving the desired degree of brush control.

Figure 25. In contrast, the standard mixture of 2,4-D + 2,4,5-T at a rate of 3 lb/A (1½ lb + 1½ lb), also applied in the fall, gave satisfactory control of brush.

Fig. 26. Partial control of brush along 800 N in Nobel County at the Kosciusko County line with Banvel 720 (a mixture of dicamba and 2,4-D) applied as a low oil + water mixture on September 17, 1973. Photographed May 21, 1974.

Fig. 27. Treatment as in Fig. 26 showing partial control of ash. Even with partial control, visibility may be greatly enhanced.

Fig. 28. Small test plot in Kosciusko County treated with Krenite Brush Control Agent. Brush is controlled but grass survives.

Fig. 29. Small test plot in Kosciusko County treated with the soil sterilant material Hyvar X-L. Brush is controlled but grass is killed leaving the soil open to erosion.

Performance comparisons. The various materials evaluated are summarized in Table 22. Neither the 3-WAY PHENOXY-DICAMBA MIXTURE nor the KRENITE BRUSH CONTROL AGENT are as effective in the control of brush as the herbicide 2,4,5-T alone or in combination with 2,4-D (BRUSH KILLER). Both are superior to 2,4-D alone, dicamba alone, or 2,4-D in combination with dicamba. These are the only materials known to be effective for control of brush which are both commercially available (except under an experimental label) and considered to be environmentally safe.

The 3-WAY MIX is less expensive than KRENITE though both are comparable in cost. They are applied at comparable rates and both are recommended for applications in the fall. The 3-WAY MIX may give the broadest spectrum of species controlled. KRENITE has a slight edge in overall safety but has not been evaluated in terms of weed control. It is not expected to be very effective.

In summary, KRENITE BRUSH CONTROL AGENT may have considerable merit for use along county roads where the species to be controlled are susceptible to KRENITE and where emphasis is on "trimming". Where a broader spectrum of control is desired, i.e. control of both borad-leaf weeds and brush along the Interstate System, then the 3-WAY MIX is recommended for application in the fall and/or spring after crops are made or before crops are up. Because of possible injury to soybeans, the 3-WAY MIX is not recommended for use along county roads in midsummer.

Table 22. Performance comparisons of brush control agents based on 1973-1976 test evaluations.

Species	BRUSH KILLER* (2,4-D + 2,4,5-T) 3 lb/A	3-WAY MIXTURE (D + banvel + Silvex) 3 lb/A	3-WAY MIXTURE (D + banvel + Silvex) 6 lb/A	KRENITE BRUSH CONTROL AGENT 6 lb/A	M-3766 (4:1 Tordon + 2,4-D) 1 lb/A	M-3766 (4:1 Tordon + 2,4-D) 2 lb/A
Ash	3	3	4	3	1	2
Elm	2	5	3	4	4.5	3.5
Maple	5	2	2	3	4	5
Locust	5	5	5	5	1-5	2-5
Willow	4	3	4	4	4	5
Other Softwoods	2-5	4	4.5	3	4	4
Oak	5	2	2.5	3	insufficient	
Other Hardwoods	5	2	2.5	4	data	
Multiflora Rose	0	4	5	2	5	5
Other brambles and vines	4	1	2	2	4	4
ALL SPECIES	3.6	3.1	3.4	3.3	3.4	4.0

* Includes data from previous evaluations

Table 22 (Contd.) Performance comparisons of brush control agents based on 1073-1976 test evaluations.

Species	SILVEX* (2,4,5-TP) 3 lb/A	6 lb/A	BANVEL (Dicamba) 2 lb/A	4.4 lb/A	BANVEL 720 (Dicamba + 2,4-D) 3 lb/A
Ash	0	2.5	4	4	4
Elm	4	5	2	3	2
Maple	1	2	0	0	0
Locust	5	5	variable		2
Willow	2	3	3	4	2
Other Softwoods	3	3	2	2	2
Oak	3	4	3	1	0
Other Hardwoods	3	3	3	3	0
Multiflora Rose	2	2	0	0	2
Other brambles and vines	3	3	1	1	1
ALL SPECIES	3.0	3.3	1.8	2.1	1.5

* Includes data from previous evaluations.

3-WAY PHENOXY-DICAMBA HERBICIDE MIXTURE

CHEMICAL: A mixture of equal parts of an amine salt formulation of 2,4-D (2,4-dichlorophenoxyacetic acid) + 2,4,5-TP (Silvex = 2,4,5-trichlorophenoxypropionic acid) + dicamba (Banvel = 3,6-dichloro-o-anisic acid)

RATES are based on a tank mix of formulated products each containing 4 pounds per gallon of active material.

DIRECTIONS FOR USE

For the control of woody brush (susceptible) including:

- Ash
- Basswood
- Blackberry
- Black Locust
- Box Elder
- Catalpa
- Cherry
- Chinquapin Oak
- Crab Apple
- Dogwood
- Elm; Elderberry
- Honey Locust
- Multiflora Rose
- Oak
- Persimmon
- Poplar
- Sassafras
- Sumac
- Sycamore
- Wild Cherry
- Wild Plum
- Wild Grape
- Wild Rose
- Willow
- Witchhazel

and similar woody plant species

HOW TO MIX

1 gallon 2,4-D amine
+ 1 gallon Silvex
+ 1 gallon dicamba plus
300 gallons water

2½ gallons 2,4-D amine
+ 2½ gallons Silvex
+ 2½ gallons dicamba plus
92.5 gallons of water

HOW TO APPLY

HYDRAULIC SPRAY APPLICATION
STEM-FOLIAGE-HIGH WATER VOLUME

Apply after leaves are fully developed and until about three weeks before frost.

Treat all stems and foliage to runoff including root crown, if possible

Use 100 to 150 gallons of spray mix per acre depending on the height and density of brush

AERIAL APPLICATION OR GROUND APPLICATION WITH MICROFOIL BOOM

Apply after leaves are fully developed and until about three weeks before frost

Apply at the rate of 20 gallons of spray mix per acre of brush

Also controlled (intermediate):

- Bittersweet
- Cottonwood
- Hawthorne (Red Haw)
- Hickory
- Maple
- Mulberry
- Walnut

Not controlled (resistant):

- Hackberry
- Greenbriar

"KRENITE" BRUSH CONTROL AGENT

CHEMICAL: Krenite (ammonium ethyl carbamoylphosphonate) plus a NONIONIC SURFACTANT (Surfactant WK, Tween 20, or Triton X-100 or equivalent product)

RATES are based on a tank mix of formulated product containing 4 pounds per gallon of active material plus a surfactant.

DIRECTIONS FOR USE

For the control of woody brush (susceptible) including:	HOW TO MIX	HOW TO APPLY
Alianthus Aspen Beech Birch Hawthorne Hornbean Locust (Black & Honey) Multiflora Rose Red Oak White Oak Water Oak Sycamore Sumac Sweet Gum Walnut Blackberry and similar woody plant species	1½ - 3 gallons Krenite + 1 quart surfactant plus 100 gallons water (or follow manufacturer's label instructions)	HYDRAULIC SPRAY APPLICATION STEM-FOLIAGE-HIGH WATER VOLUME Apply from August 1 until September 15. Treat all stems and foliage to runoff. Good coverage is critical. Use 150 to 200 gallons of spray mix per acre depending on the height and density of brush.
	2½ - 3 gallons Krenite + ½ pint surfactant plus 17 - 17½ gallons water (or follow manufaeturer's label instructions)	AERIAL APPLICATION OR GROUND APPLICATION WITH MICROFOIL BOOM Apply from August 1 until September 15

Also controlled (intermediate):

Red Alder
White Ash
Black Cherry
Choke Cherry
Pin Cherry
Dogwood
Elderberry
American Elm
Slippery Elm
Winged Elm
Black Gum
Hackberry
Hickory
Red Maple
Mulberry
Osage Orange

Apply at the rate of 20 gallons of spray mix per acre of brush.

Persimmon
Poison Ivy
Sassafras
Smilax (Green Briar)
Virginia Creeper
Wild Grape
Wild Plum
Willow
Witchhazel
Yellow Poplar

Not controlled (resistant):

Box Elder
Buckeye
Buckbrush
Basswood
Eastern Red Cedar
Honeysuckle
Linden
Russian Olive
Trumpet Creeper

ENVIRONMENTAL SAFETY AND SPECIAL PRECAUTIONS

3-WAY PHENOXY-DICAMBA MIXTURE: The components of this mixture all meet current regulatory requirements for use on land adjacent to and surrounding domestic water supplies, streams, lakes and ponds as well as for roadsides.

2,4-D is registered for roadside use without restriction. When amine forms 2,4-D are used, there is no danger to fish, other wildlife, domestic animals, or phytoplankton. The acute mammanian toxicity is low and 2,4-D poses no known hazards to humans. There is no danger to non-target vegetation or crops unless directly oversprayed. 2,4-D is biodegradable, with a soil-water persistence half-life of about 4 days.

Dicamba is registered for roadside use with no restrictions. It is non-toxic and also biodegradable with a soil-water persistence half-life of 25 days. It is not toxic to fish or phytoplankton or other forms of aquatic life. The acute mammalian toxicity is low and dicamba does not pose any known hazards to humans. There is some potential hazard to non-target vegetation, especially susceptible species including soybeans, from fine spray particles carried in the form of drift. Important: WHEN THE 3-WAY MIXTURE CONTAINING DICAMBA IS USED, AS A PRACTICAL MATTER DITCHES WHICH BORDER SOYBEAN FIELDS SHOULD BE SPRAYED ONLY BETWEEN AUGUST 1 and SEPTEMBER 15 (OR AFTER SOYBEANS ARE MADE) OR IN EARLY SPRING BEFORE SOYBEANS ARE UP. At other times, the dicamba in the mixture can be replaced by 2,4-D amine or by 2,4DP amine.

Silvex, unlike 2,4,5-T, can be used in roadside applications. Its toxicity properties are similar to those for 2,4-D. It is biodegradable with a soil-water persistence half-life of about 20 days. Unfortunately, Silvex is sold only as an ester formulation. Therefore, fish kills may result if rates in excess of 2 lb/A are sprayed directly over shallow water. Important: The amount of Silvex must not exceed more than 1/3 of the total herbicide in the mixture. Silvex must not be combined with ester formulations of 2,4-D ditch bank applications. The total rate of application (2,4-D amine + dicamba + Silvex) must not exceed 6 lb/A. If any of the above are violated, fish kills may result.

KRENITE BRUSH CONTROL AGENT + SURFACTANT: Krenite is labeled for use on "land adjacent to and surrounding domestic water supply reservoirs, supply streams, lakes, and ponds." There is little or no potential danger to non-target vegetation or crops unless directly oversprayed. Even with brush, unsprayed portions of larger trees normally survive. Krenite has a low mammalian toxicity, is biodegradable and poses no known hazards to humans.

The SURFACTANTS recommended for use with Krenite Brush Control Agent are not toxic to humans and generally recognized as safe. The recommended amounts should not be exceeded due to possible injury to fish.

METHODS OF APPLICATION FOR FOLIAR BRUSH CONTROL SPRAYING

Foliar applications of brush control agents is normally less expensive than dormant basal or directed applications, because less labor and time is required and water is used as the carrier. The most desirable season for foliar applications is late summer or early fall after crops are made so that dangers of injury from drift become minimal. Timing of the Krenite application is critical and must be carried out between August 1 and September 15. Timing of the 3-Way Mix application is less critical but must be between the time the brush is fully leafed out and the leaves begin turning color in the fall.

Ground application. Equipment of various types can be used with any type of off-road equipment able to traverse the area to be sprayed. Some type of boom system is most widely used. The boomless nozzle system is often criticized because of poor coverage. However, a combination of boom and boomless nozzle arrangements are very effective. Broadcast applications can be made with a handgun, and in brush applications, a straight-stream nozzle is often used. The handgun method has the advantage of being able to direct the spray to where it appears to be most needed and to reach plants not directly accessible to ground equipment. Selective treatment of brush is also possible.

The greatest problems in ground applications are to achieve adequate coverage and to avoid drift. All leaves and bark surfaces should be thoroughly wetted and the mixture even allowed to run down and puddle at the base of the trunk. If drift cannot be avoided, the applicator should stop spraying and wait for better weather conditions.

Aerial applications. Helicopters are normally used in making aerial applications but have not proven practical for most roadside uses. A truck-mounted microfoil boom can be used to simulate an aerial application with considerable success. Mixing and application rates with the microfoil boom are the same as for aerial applications. A drift-control system must be used to ensure proper application and to reduce the dangers of drift.

PROPER CONTROL OF DRIFT IS ESSENTIAL

Drift is the term used for the movement of spray particles from the time they leave the application equipment until they hit the ground. Drift damage can be avoided in the following ways:

1) Keep fine spray particles at a minimum
2) Do not spray in windy weather
3) Keep equipment and spray nozzles close to target
4) Make early or late season applications when susceptible crops are dormant or not growing
5) Utilize special drift reduction techniques such as invert emulsions, thickeners, gels, or foams.

Basically, drift can best be controlled by the elimination of fine particles in the spray (lower pressures and increased spray volume; use of larger nozzle tips), proper conditions for application (wind is the major factor), and the use of drift control agents. No drift control system is perfect, and none will control drift under windy conditions. DRIFT IS BOTH WASTEFUL OF CHEMICAL (It reduces treatment effectiveness since less chemical reaches the target) AND IS A HAZARD TO NON-TARGET SPECIES.

RECOMMENDATION

To prevent growth of brush in unmowed portions of rights-of-way along the Interstate System, a brush control agent should be added to the Fall-Spring Spraying Rotation. For this purpose, the 3-WAY PHENOXY-DICAMBA HERBICIDE MIXTURE should be used as a general broadcast treatment at the rate of about 3 lb total active material per acre as a tank mix in all "off road" spraying equipment.

Materials:

a) 2,4-D amine form concentrate containing 4 pounds of acid equivalent per gallon.

b) Dicamba amine form concentrate (Banvel) containing 4 pounds of acid equivalent per gallon.

c) 2,4,5-TP low volatile ester concentrate (Silvex) containing 4 pounds of acid equivalent per gallon. Silvex is available commercially only as an ester formulation.

Rate: The materials are to be mixed at the rate of 1 gallon of 2,4-D concentrate + 1 gallon Dicamba concentrate + 1 gallon 2,4,5-T (Silvex) concentrate in 150 gallons of water. The mixture is to be applied at the rate of 40-50 gallons per acre.

How to Apply: For best results the material should be applied as a broadcast application for control of broadleaf weeds, CANADA THISTLE, and small brush. Larger brush and thistle growth near fence line should receive, in addition, a directed spray from a hand gun to thoroughly wet leaves and stems.

Schedule of Application:

a) Fall: August 15 to September 15 (preferred time for single application)

b) Spring: Just after brush is leafed out until soybeans are up. April 1 to April 30.

COVER DESIGN BY ALDO GIORGINI